University of Alberta

Characterization of Gas Oil Resins using
High Performance Liquid Chromatography

by

Patricia Ann Hilario Arboleda ©

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"If I have seen a little further

it is by standing on the shoulders of Giants."

Isaac Newton in a letter to Robert Hooke

February 5, 1676

This thesis is dedicated to my husband

ABSTRACT

Petroleum gas oil resins are polar species that cause catalyst deactivation and fouling in refineries. With their large boiling range (204 – 524°C), complexity of polyaromatic and heteroaromatic species, and tendency to adsorb to GC columns, the best possibility of separation is by High Performance Liquid Chromatography (HPLC).

In this thesis, three HPLC columns are evaluated using model compounds, including gel permeation chromatography (GPC), silica-based amino-cyano (SAC), and hypercrosslinked polystyrene (HCP). GPC had limited success. SAC gave better separation but HCP had the best separation. Therefore, four different gas oil resins were separated using HCP. The separation required a four-solvent gradient, resulting in seven peaks in 35 minutes. Based on model compounds, a separation between aromatics, and sulfur- and nitrogen-containing compounds was achieved.

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LIST OF SYMBOLS AND ABBREVIATIONS

au	absorption unit
BP	boiling point
C_6	hexane
$CDCl_3$	deuteriochloroform
$CHCl_3$	chloroform
C_m	concentration in the mobile phase
C_s	concentration in the stationary phase
d_c	column internal diameter
d_p	packing particle diameter
ε	molar absorptivity or extinction coefficient
F	mobile phase flow rate
FBP	final boiling point
GC	gas chromatography
h	reduced plate height
H	column plate height (HETP value)
HPLC	high performance liquid chromatography
HTSD	high-temperature simulated distillation
IBP	initial boiling point
ID	internal diameter
k'	retention factor
kPa	kilo Pascal
L	length of column
LC	liquid chromatography
MeOH	methanol
MW	molecular weight
N	column plate number or efficiency
NMR	nuclear magnetic resonance
NPLC	normal phase liquid chromatography
P	time from width start point at 5% peak height to retention time

ppm	parts-per-million concentration
ppt	parts-per-thousand concentration
psi	pound per square inch
QNLC	quasi normal phase liquid chromatography
r	column radius
R_f	response factor (au/ppm)
RPLC	reverse phase liquid chromatography
R_s	resolution
RSD	relative standard deviation
Si	silica
T	temperature
THF	tetrahydrofuran
T_f	tailing factor
t_o	column dead time
t_r	retention time
t_v	retention volume
USP	United States Pharmacopeial
vol%	volume percent
V_E	total exclusion volume
V_m	volume of stationary phase
V_o	void volume
V_P	total permeation volume
V_s	volume of sample loop
$W_{0.5}$	width at half-height
$W_{5\%}$	peak width at 5% peak height
W_B	width at baseline
wt%	weight percent concentration
η	viscosity
λ	wavelength in nanometers
λ_{max}	wavelength at which maximum absorption in the spectrum (nm)

Å	Angstrom
α	separation factor or separation selectivity
ΔP	column pressure drop

Chapter 1. Introduction

1.1 Motivation and thesis overview

Petroleum[1] is a complex mixture of components [1]. One method of characterizing petroleum is distillation[2]. For example, heavy oil[3] or bitumen[4] [2-4] is subjected to distillation (Figure 1.1) [1,5] to yield gas oil, comprising of light gas oil (LGO) and heavy gas oil (HGO).

Gas oil contains materials that can deactivate (or "poison") the catalysts used in refinery processes, or cause congestion of the distillation tower [4]. Chemical components of gas oil can also influence its processability by stabilizing emulsions[5] and sludges[6] [7-10]. Therefore, it is desirable to characterize the composition of gas oil and detect components that will influence the refining processes.

Gas oil can be characterized in terms of solubility into saturates[7], aromatics and gas oil resins fractions. Gas oil is dissolved in n-pentane and eluted through preparative chromatography[8] using a column packed with clay in the upper section and a mixture of silica gel plus clay in the lower section (Figure 1.2). A fraction containing saturated hydrocarbons is eluted using n-pentane, then a fraction of aromatic compounds is eluted using 50:50 (vol%) n-pentane:toluene, and finally the gas oil resins fraction is eluted using 50:50 (vol%) toluene:acetone [11] (Figure 1.2).

---

[1] Petroleum: A substance, generally liquid, naturally occurring in the earth and composed mainly of carbon and hydrogen with some sulfur, oxygen, and nitrogen. Examples of petroleum are heavy oil and bitumen.
[2] Distillation: Distillation method is the separation of substances based on differences in their volatilities.
[3] Heavy Oil: Petroleum with density of > 0.9 g mL^{-1}.
[4] Bitumen: Petroleum with density of > 0.96 g mL^{-1}.
[5] Emulsion: A mixture of oil in water or water in oil.
[6] Sludge: A mixture of oil, water, and sediment that forms due to petroleum incompatibility.
[7] Saturates: Saturated hydrocarbons ex: n-alkanes, branched alkanes and cyclic alkanes.
[8] Preparative chromatography: Preparative chromatography is the larger scale chromatography involving gram to kilogram sample size. Open or closed columns maybe used for sample cleanup and preconcentration.

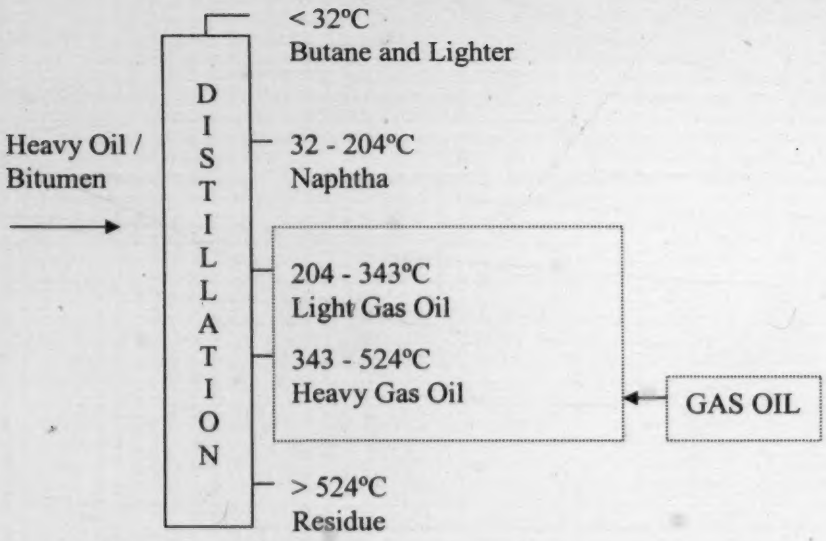


Figure 1.1 An example of a petroleum refining layout. The products are determined based on boiling point (°C). Adapted from references [1,6,12].

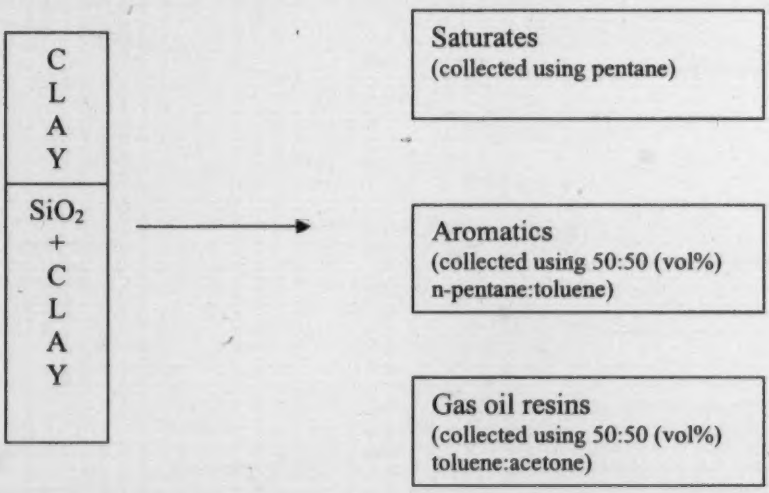


Figure 1.2 Gas oil undergoes preparative chromatography to extract saturates, aromatics and gas oil resins [11].

In the preparative chromatography method for gas oil (Figure 1.2), the total weight percent saturates, aromatics and resins are determined [11]. Saturates and aromatics can be further analyzed using gas chromatography - mass spectrometry (GC-MS), a technique which offers high resolving power with approximately 100,000 theoretical plates[9] and can effectively separate materials up to 430°C [1,5]. In a GC-MS analysis, gas oil resins are un-resolved and are highly retained. Therefore, gas oil resins are subjected to no further analysis.

Gas oil resins have large boiling point range (204 – 524°C), contain large aromatic compounds (up to 5 aromatic rings) and have high sulfur and nitrogen content (>1%). High performance liquid chromatography (HPLC) offers an advantage over GC in that the analytes do not have to be volatile. Therefore, HPLC is a good candidate for separating gas oil resins.

Currently, standardized HPLC and supercritical fluid chromatography methods for analyzing petroleum products are limited to middle distillates (150 – 400°C) containing little (<1%) to no polar components [11,13,14]. Therefore there is a need to find a column that will be able to separate gas oil resins.

Gas oil resins contain up to 5 aromatic rings. They also contain sulphur, in the form of thiophene [1,15-18], sulfides and thiols [19-21]. Determining the distribution of sulphur compounds among these groups would aid in determining which sulphur bridges break down more easily on desulphurization [1]. In the petroleum industry, nitrogen compounds are classified as basic, neutral or acidic [1]. The basic compounds are pyridines and amines. The neutral, or weakly basic compounds are alkylindoles, alkylacridine, some amides and alkylhydroxypyridines. Pyrroles, such as indoles and carbazoles, are acidic. Acidic nitrogen species in particular, are involved in catalyst deactivation during refinery processes and implicated in fouling and corrosion of refinery units [22]. Consequently, it is important to be able to quantify these species. Overall, it is desirable to determine the distribution of sulphur and nitrogen compounds in gas oil resins in order to determine their role in fouling[10] and corrosion[11] [23].

---

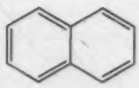
[9] Theoretical Plates: A number that describes the ability of a column to give narrow bands. The larger the number, the better it is at resolving peaks.

[10] Fouling: Fouling is the undesirable accumulation of carbonaceous inside tubing or pipes, and inside other components, such as pumps, compressors, and heat exchangers.

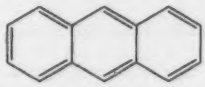
In this thesis, three HPLC columns are studied for gas oil resins application. Three separation methods are explored. The first is separation by size and shape using gel permeation chromatography. The second is normal phase liquid chromatography using silica-based amino-cyano column. The third is quasi-normal phase mode using a hypercrosslinked polystyrene stationary phase. Model compounds are eluted in all columns to determine column performance (Figures 1.3 and 1.4). Finally, gas oil resins from different sources are analyzed.

Aromatic: Linear

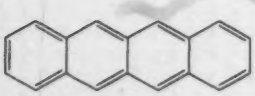
1.3.1 benzene



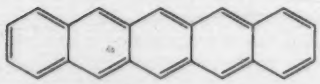
1.3.2 naphthalene



1.3.3 anthracene

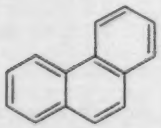


1.3.4 tetracene

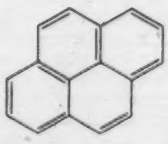


1.3.5 pentacene

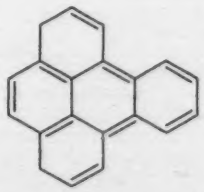
Fused angled



1.3.6 phenanthrene



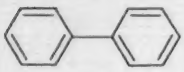
1.3.7 pyrene



1.3.8 benzo(e)pyrene

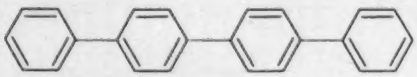
[11] Corrosion: Corrosion is the deterioration of a material due to its environment. Acidic species in petroleum remove iron from pipes causing equipment failure.

Biphenyl linked



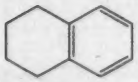
1.3.9 biphenyl

1.3.10 p-terphenyl

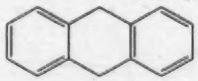


1.3.11 p-quaterphenyl

Dihydroaromatic



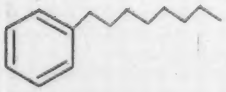
1.3.12 tetralin



1.3.13 9,10-dihydroanthracene

1.3.14 5,12-dihydrotetracene

Phenyl branched



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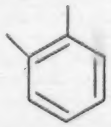
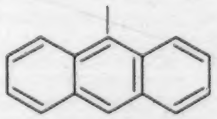


1.3.16 o-xylene

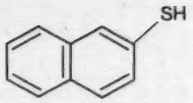


1.3.17 9-methylanthracene

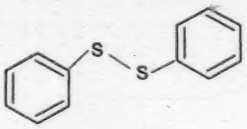
Non-aromatic

1.3.18 cyclohexane

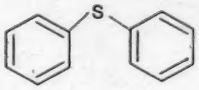
Figure 1.3 Structures of aromatic and non-aromatic model compounds.



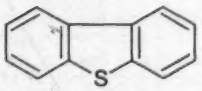
1.4.1 2-naphthalenethiol



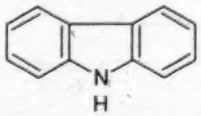
1.4.2 phenyldisulfide



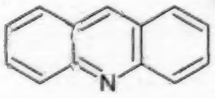
1.4.3 phenylsulfide



1.4.4 dibenzothiophene

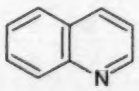


1.4.5 carbazole

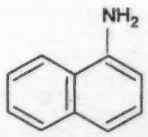


1.4.6 acridine

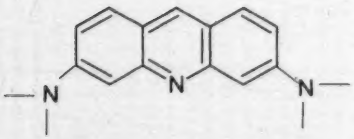
1.4.7 pyridine



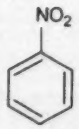
1.4.8 quinoline



1.4.9 2-aminonaphthalene



1.4.10 acridine orange



1.4.11 nitrobenzene

Figure 1.4 Structures of aromatic sulphur- and nitrogen-containing model compounds.

1.2 T-test

In this thesis the optimum HPLC method for the separation of gas oil resins is being determined. Thus, throughout this thesis, experiments are compared to determine the most advantageous method. A statistical tool used to compare results from different experiments is a t-test [24,25]. A t-test is used to compare one set of measurement with another to determine if they are different. The t-test assumes that data points are clustered symmetrically about the average value of a Gaussian distribution and in this thesis, the data points will be found within 95% confidence interval. A value $t_{calculated}$ is computed and then compared to a predetermined value appropriate for the specific conditions, t_{table} [24,25], to determine whether the change is statistically significant.

For comparing measured values to known values the value of $t_{calculated}$ is computed according to the equation:

$$t_{calculated} = \frac{|\,knownvalue - x_{average}\,|}{s}\sqrt{n} \qquad (1.1)$$

In Section 4.3.2.2, three (n = 3) measurements of the total peak area eluting from the column was made, obtaining an average value ($x_{average}$) and a standard deviation (s) (Equation 1.2). The measured peak areas were compared to the known amount of sample injected onto the column (known value).

$$s = \sqrt{\frac{\sum(x - x_{average})^2}{(n-1)}} \qquad (1.2)$$

The t_{table} for 3 measurements with 2 degrees of freedom and a 95% confidence interval can be found in references [24,25]. If $t_{calculated}$ is less than t_{table} then the measured values and known values are statistically equivalent. If $t_{calculated}$ is greater than t_{table} then the measured values and known values are different at the 95% confidence limit.

For comparing replicate measurements from different methods the value of $t_{calculated}$ is computed according to the equation:

$$t_{calculated} = \frac{X_{1average} - X_{2average}}{S_{pooled}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \qquad (1.3)$$

For example, in Section 4.3.2.4 the efficiency was determined for four injections (n_1 and n_2) for each of the two different methods that gave two different averages ($X_{1average}$ and $X_{2average}$) and different standard deviations (s_1 and s_2). The S_{pooled} is the pooled standard deviation of both sets of data given by:

$$S_{pooled} = \sqrt{\frac{s_1^2(n_1 - 1) + s_2^2(n_2 - 1)}{n_1 + n_2 - 2}} \qquad (1.4)$$

The t_{table} for 4 measurement with 3 degrees of freedom and a 95% confidence interval can be found in references [24,25]. If $t_{calculated}$ is less than t_{table} then the two methods agree within experimental error.

For comparing individual differences $t_{calculated}$ is computed according to the equation:

$$t_{calculated} = \frac{d_{ave}}{s_d} \sqrt{n} \qquad (1.5)$$

For example, in Section 3.3.3, carbon number 14 of linear aromatic and fused angled (Figure 1.3) differed in retention factor (k'). The same goes for carbon numbers 18 and 22. The average difference (d_{ave}) between linear and fused angled aromatics for each pair (n = 3) was calculated and the sample standard deviation (s_d) is given by:

$$s_d = \sqrt{\frac{\sum (d_i - d_{ave})^2}{n - 1}} \qquad (1.6)$$

The t_{table} for 3 data points with 2 degrees of freedom and a 95% confidence interval can be found in references [24,25]. If $t_{calculated}$ is less than t_{table} then the two methods agree within experimental error.

1.3 High Performance Liquid Chromatography

1.3.1 Chromatographic terms

The word "chromatography" comes from the Greek words "chroma" (color) and "graphein" (to write) and was coined by Mikhail S. Tswett (1872 – 1919) during his research on chlorophyll, where he isolated plant pigments using a liquid-adsorption column containing calcium carbonate [26-28]. (It is interesting to note that Tswett's name is similar to the Russian word for color, "tsvet".) Since then the term chromatography has been used to describe the separation of a mixture into different components by passing it through a stationary phase.

The pioneers of chromatography include Martin and Synge, who were awarded the 1952 Nobel Prize in Chemistry [29] for their work on partition chromatography [30], and Van Deemter and Giddings, who developed rate theory of separation efficiency that predicted the higher separation efficiency of smaller stationary phase particles [31,32]. In the 1960's, Horvath and associates developed high pressure pumping systems to accommodate the high backpressures generated by these small particles. This was the origin of high performance liquid chromatography (HPLC) [33,34].

An HPLC system consists of a reservoir of degassed solvent, one or more high-pressure eluent pumps, an injector, a column packed with stationary phase, a detector, and a computer for data collection (Figure 1.5). The solvent is placed in a reservoir to be degassed[12] and isolated from contact with air. The solvent is forced through the

[12] Degassing: Degassing is the practice of removing air from the mobile phase. It is achieved by helium sparging, applying a vacuum to the mobile phase, ultrasonification, or heating. It is important to degas to reduce bubble formation when solvents are mixed and also to increase sensitivity and reduce baseline noise, particularly at low UV wavelengths.

system by a pump and becomes the liquid mobile phase. A reproducible volume of the analytes to be separated is introduced into the system by an injector. The liquid mobile phase carries the analytes through a column containing a solid stationary phase. The analytes separate based on different rates of travel through the column. Separation will occur if the analytes interact differently with the column. A detector measures the concentration of each analyte band as it leaves the column. When a sample band reaches the detector, the detector responds, giving rise to a change in detector signal, seen as a peak. The detector signal is recorded in a computer as a chromatogram[13]. Ideally all the analytes are resolved.

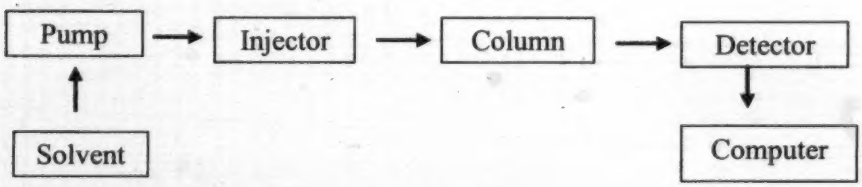


Figure 1.5 Typical HPLC instrumental scheme.

Resolution is a quantitative measure of how well two analytes are separated in a chromatographic analysis, as per the equation:

$$R_s = \frac{2(t_2 - t_1)}{(W_{b1} + W_{b2})} \qquad (1.7)$$

where R_s is resolution, t_2 and t_1 are the retention times of two peaks corresponding to different analytes, and W_{b1} and W_{b2} are the width at baseline of each peak (Figure 1.6).

[13] Chromatogram: A chromatogram is a plot or record of a voltage signal as a function of time.

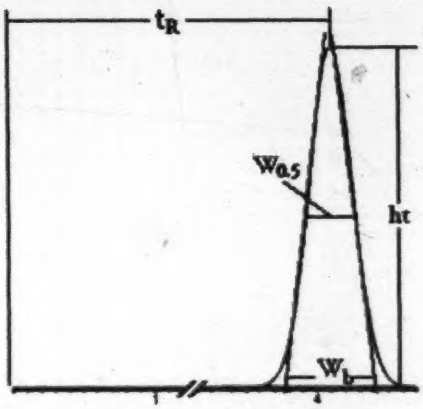


Figure 1.6 The width at tangent (W_b) and width at half-height ($W_{0.5}$). Adapted from reference [35].

The baseline width at tangent will increase as the peak tails or broadens. Good resolution is obtained when the peaks have very different elution times (t_2-t_1) and are not very broad (W_{b1}+W_{b2}). Two peaks are said to be "baseline resolved" if R_s is greater than or equal to 1.5. During method development the critical peaks (adjacent peaks with the smallest resolution) are targeted to have a resolution of at least 1.5 [36].

Resolution can also be calculated using the width at half height ($W_{0.5}$) (Figure 1.6) as per:

$$R_s = \frac{2(t_2 - t_1)}{1.7(W_{0.5,1} + W_{0.5,2})} \qquad (1.8)$$

This method of calculating resolution is not as sensitive to tailing or peak broadening, and so it may overestimate R_s. Most computer HPLC programs automatically calculate width at half height. It is important to state which equation is being used (width at tangent or width at half height) as the two values are unequal. In this thesis, it will be specifically stated which equation is being used in each section.

The factors that affect resolution are the retention factor (k'), selectivity (α), and efficiency (N).

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{k'+1}\right) \qquad (1.9)$$

Retention time is the time it takes for a compound to elute out from the column, and will vary according to column dimensions and mobile phase flow velocity. The retention factor (k') is a more consistent measure because it cancels the effects of column dimensions and mobile phase flow rate. The retention factor is calculated from the retention time of the peak (t_r) and the retention time of an un-retained solute (t_o) (Figure 1.7). The retention factor can be adjusted by modifying the mobile phase composition [36].

$$k' = \frac{(t_r - t_o)}{t_o} \qquad (1.10)$$

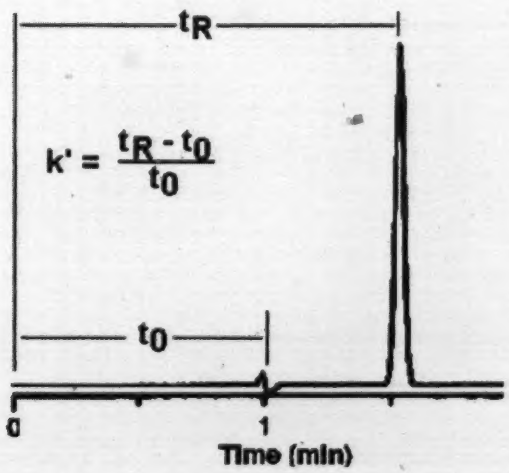


Figure 1.7 The retention factor, k'. Adapted from reference [35].

The retention time of an un-retained solute (t_o) can be approximated using equation [37]:

$$t_o \cong \frac{0.5Ld_c^2}{F} \qquad (1.11)$$

where L is the length of the column (cm), d_c (cm) is the diameter of the column and F ($cm^3\ min^{-1}$) is the flow rate.

Selectivity (α) is the ability of the system to discriminate between two analytes eluting close together. It is the ratio of the retention factors (k') of the two adjacent peaks.

$$\alpha = \frac{k'_2}{k'_1} \qquad (1.12)$$

The selectivity is dependent on the solvent and column types [36].

Efficiency (N), also known as the number of theoretical plates, is a measure of the broadening of a peak. It accounts for the dispersion a peak undergoes while traveling through the system. Assuming a Gaussian peak shape, N is calculated using the width at baseline (W_b, Figure 1.6) and the retention time of the peak (t_r, Figure 1.7).

$$N = 16\left(\frac{t_r}{W_b}\right)^2 \qquad (1.13)$$

Efficiency can also be calculated based on the width at half-height ($W_{0.5}$).

$$N = 5.54\left(\frac{t_r}{W_{0.5}}\right)^2 \qquad (1.14)$$

The tailing factor (T_f) as per United States Pharmacopeial (USP) is a measure of how symmetrical a peak is, where:

$$T_f = \frac{W_{5\%}}{2 \times P} \qquad (1.15)$$

$W_{5\%}$ is peak width at 5% peak height, and P is time from width start point at 5% peak height to retention time. For a symmetrical peak, the tailing factor, T_f, is 1.0, and the value of T_f increases as tailing becomes more pronounced.

The number of theoretical plates (N) can be increased by increasing the length of the column (L) or by decreasing the column plate height (H).

$$N = \frac{L}{H} \qquad (1.16)$$

Column plate height (H) is a measure of peak broadening. Van Deemter's rate theory [31] identifies three factors that contribute to the column plate height: eddy diffusion (A), longitudinal molecular diffusion (B), and resistance to mass transfer (C).

$$H = A + \frac{B}{u} + Cu \qquad (1.17)$$

In this equation, u is the linear velocity of the mobile phase. Eddy diffusion (A) represents the fact that a molecule can take many different paths of different length as it travels through the column. A tightly packed column containing small-diameter particles will minimize eddy diffusion. The B term refers to longitudinal diffusion as the molecules diffuse from regions of high concentration to lower concentration over time. According to Van Deemter's rate theory, using high flow velocity can minimize the contribution of this term to peak broadening. At the flow rates typically used in HPLC, longitudinal diffusion is generally negligible. Resistance to mass transfer (C) is the transfer of analyte molecules in and out of the stationary phase. Decreasing the flow velocity minimizes the C term. Increasing the temperature of the column, decreasing particle diameter, and use of a thinner stationary phase film minimize C by

ensuring fast transfer in and out of the stationary phase. Overall, resolution can be fine-tuned by changing the type of column, column length, stationary phase particle diameter, uniformity of particle size and pores, mobile phase composition, mobile phase flow rate, and temperature [36].

1.3.2 Modes of separation

High performance liquid chromatography (HPLC) is a form of column chromatography used in analytical chemistry. HPLC separates mixtures into different components based on differences in the interactions between the analyte and the chromatography column, as well as the interactions between the analyte and mobile phase. Several of these modes of separation will be described in the following sub-sections.

1.3.2.1 Normal phase liquid chromatography

Normal phase liquid chromatography (NPLC) is the mode of separation used in the earliest HPLC experiments of Tswett. Analytes are retained based on polarity, using a polar stationary phase with a non-polar mobile phase. This mode is used for the separation of polar analytes, carbohydrates, and other uncharged hydrophilic species [36]. Petroleum, on the whole, is non polar. But as described in Section 1.1, it can contain polar components where NPLC is the preferred separation mode primarily due to the solubility and compatibility of fossil fuel-derived samples with normal-phase solvents [5].

Retention in NPLC is based on the adsorption of polar functional groups within on the analyte molecules onto polar sites on the column packing. The most polar analytes elute last. The predominant interactions in NPLC are dipole-dipole and dipole-induced-dipole; depending on the analyte, hydrogen bonding can also occur [38]. Columns used for petroleum analysis by NPLC are typically packed with silica stationary phase or silica with added polar functional groups such as amino, cyano and diol [39,40], as well as dinitroaminopropyl [41-43]. Typical solvents used for the

mobile phase are hexane, heptane, dichloromethane, [redacted] acetate, methyl-t-butyl ether, isopropyl alcohol and tetrahydrofuran [36].

1.3.2.2 Reversed phase liquid chromatography

Reversed phase liquid chromatography (RPLC) is an HPLC separation mode that is widely used in pharmaceutical, protein and environmental applications [44]. It uses a non-polar stationary phase and a polar mobile phase. RPLC columns consist of silica particles to which hydrocarbons such as C_8H_{17} and $C_{18}H_{37}$ are bonded. The predominant interaction is London dispersion forces [36]. More hydrophilic (polar) compounds elute first and more hydrophobic (non-polar) compounds elute last. Typical mobile phases are water, acetonitrile and methanol [36].

1.3.2.3 Isocratic liquid chromatography

In isocratic liquid chromatography, a mobile phase of constant composition is used. This is in contrast to gradient liquid chromatography (1.3.2.4), in which the composition of the mobile phase changes as the separation proceeds. Some systems, such as size exclusion chromatography, must be performed in isocratic mode to ensure mobile phase compatibility with the column [5,45-47]. In general, if all components elute with retention factors in the range $2<k'<10$, isocratic mode will suffice [36].

In the petroleum industry, isocratic methods have been applied to light and middle distillates, and usually involve back-flushing[14]. American Society of Testing and Materials (ASTM) [14] and Institute of Petroleum (IP) [13] methods recommend amino-silica or aminocyano-silica column and isocratic hexane or heptane mobile phases to analyze samples with boiling points in the range 150 – 400°C. This method elutes saturates, mono-aromatics, and di-aromatics, with the poly-aromatics eluted by back-flushing the column [13,14]. Suatoni and Garber [48] have used silica columns

[14] Back-flushing: At a set-time the direction of solvent flow through the column is reversed. Back-flushing is used to elute late eluting components or analytes with low concentration.

and n-hexane to separate saturates, mono-olefins[15], and di-olefins, with the aromatics eluted by back-flushing. Hayes and Anderson [49] have used silica gel impregnated with silver nitrate and an isocratic mobile phase of 2,2-dichloro-1,1,1-trifluoroethane (Freon-123) to elute saturates and aromatics, with back-flushing to remove olefins from the column.

1.3.2.4 Gradient liquid chromatography

In gradient elution, the composition of the mobile phase is systematically changed during a separation process. Gradient elution is applied to mixtures of components with a wide range of retention factors (k'). It can be used for analytes present in low concentrations that elute late in the separation [36]. Gradient elution is also useful for identifying a suitable solvent composition when performing preliminary work on an unknown sample [36,44].

Group type separations[16] of petroleum are performed using NPLC with gradients, back-flushing, valves, and multiple columns. For example, Grizzle and Sablotny [50] used amino-bonded silica columns and an n-hexane/dichloromethane gradient mobile phase. Saturated hydrocarbons and aromatics were eluted, and the polar compounds were back-flushed. Robbins [43] used three solvents, propylamino-cyano and dinitro-aminopropyl columns to analyze a petroleum fraction with a boiling point up to 700°C. The mobile phase consisted of n-hexane, dichloromethane, and 2-propanol, with valves to control to which column the sample flow through. This method eluted saturates, 1-4 ring aromatics and polar compounds. Padlo *et al.* [51] used a gradient mobile phase composed of n-pentane, dichloromethane and 2-propanol. Valves were used to control to which column: amino-cyano, dinitroaminopropyl-Si and diol-Si stationary phases, the samples flow through. Saturates, 1-4 ring aromatics and polar compounds were eluted.

---

[15] Olefins: Unsaturated hydrocarbon containing at least one carbon to carbon double bond (alkene).
[16] Group type separation: Separation into different classes of compounds. For example, separate saturated hydrocarbons, mono-aromatic, di-aromatic, and poly-aromatic.

1.3.2.5 Quasi-normal phase chromatography

As described in Section 1.3.2.1, normal phase chromatography often involves a silica-based stationary phase functionalized with polar groups. The term "quasi-normal phase chromatography" (QNLC) was coined by Davankov *et al.* to describe a normal phase separation mode using a stationary phase with no specific polar groups [52]. In QNLC the predominant interactions are π- π interactions between the analyte and the stationary phase [38] such as London dispersion forces [53].

To measure π - π interaction strengths in normal phase mode, Davankov *et al.* [38] tested the selectivity factor for a separation of anthracene and phenol using a number of different stationary phases: bare silica, silica with C_8H_{17} and $C_{18}H_{37}$ reversed-phase coatings, chiralcel[17], hypercrosslinked polystyrene and porous graphitic carbon. They reported selectivity factors of 0, 0.16, and 3.41 for the bare silica, reverse-phase, and chiralcel columns, respectively, and "very large" selectivity factors for hypercrosslinked polystyrene and carbon. They found that the hypercrosslinked polystyrene and graphitic carbon stationary phases "almost irreversibly" retained anthracene in an isocratic 93:7 (vol%) hexane:2-propanol mobile phase, and concluded that of all the columns tested, hypercrosslinked polystyrene and graphitic carbon gave the strongest π - π interactions.

QNLC is best suited for the separation of aromatic compounds in non-polar matrices, either for direct liquid chromatography analysis or when the fractions from HPLC are to be subjected to further analysis by gas chromatography [54]. Typical solvents used in QNLC are pentane, hexane, dichloromethane, chloroform and 2-propanol [52].

1.3.2.6 Size Exclusion Chromatography

Size exclusion chromatography (SEC) differs from other chromatographic methods in that there should be no surface interactions between the analytes and the stationary phase. Ideally, separation is based strictly on the size and shape of the

[17] Chiralcel: A cellulose derivative adsorbed onto silica gel.

analyte molecule, and the average pore size, and packing geometry of the stationary phase [55]. The desirable characteristics of SEC include high chromatographic resolution, a linear calibration curve (i.e. there is a linear relationship between the logarithm of an analyte's molecular weight and its elution volume), and an inert and rugged stationary phase [56].

SEC separates molecules according to differences in their effective molecular sizes and shapes. The sample is introduced into a continuously flowing stream of solvent, and passes through a column packed with porous rigid-gel particles. Smaller analytes spend more time within the small pores in the stationary phase, but larger analytes cannot fit within the small pores and thus remain in the moving interstitial mobile phase (Figure 1.8). Elution order is from large to small molecules. In SEC controlled pore size is very important because the pore size determines the range of molecular weights that can be separated. Stationary phases for SEC with a wide range of pore sizes are available [45-47,55,57-59].

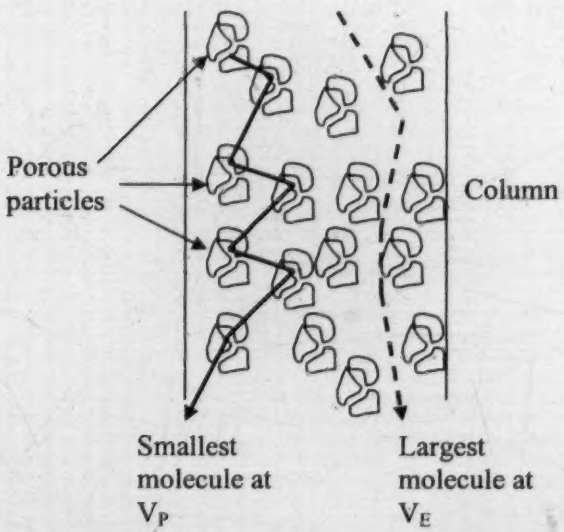


Figure 1.8 Schematic of size exclusion chromatography column. Smallest molecule elutes at total permeation volume (V_P) and largest molecule elutes at total exclusion volume (V_E).

In SEC, results are discussed in terms of elution volumes rather than retention times. Retention volume is calculated from the retention time and the mobile phase flow rate. Two elution volume limits can be defined in SEC: total exclusion and total permeation. Large molecules which pass through the column with no penetration into the stationary phase pores elute at the total exclusion limit (elution volume V_E) (Figure 1.8). Molecules small enough to fully access all of the pore volume elute at the total permeation limit (elution volume V_P) (Figure 1.8).

The V_E and V_P can be represented through a SEC calibration curve ie. a linear relationship between the logarithm of an analyte's molecular weight and its elution volume (Figure 1.9). The molecular weight of the analytes must all fall between the total exclusion and total permeation limits to be separated. Past the V_E or V_P the data points deviate from the linear trend line indicating total permeation into the pores. For accurate and optimum resolution, the slope of the calibration curve must be linear and flat as possible.

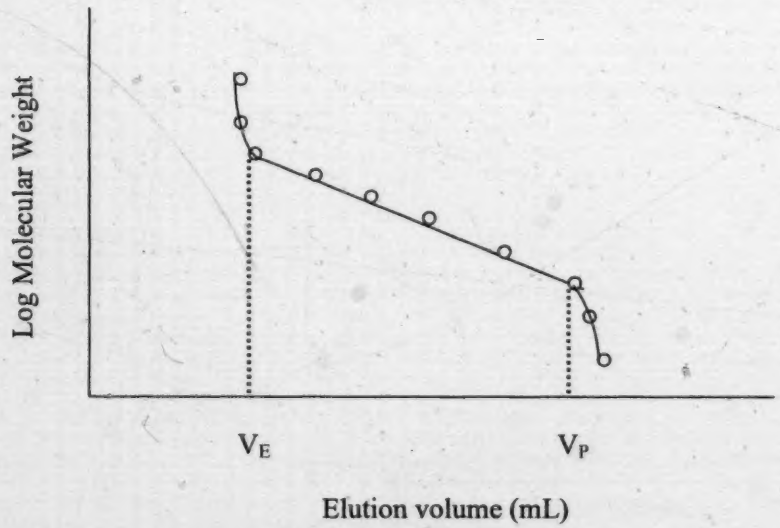


Figure 1.9 An example of a SEC calibration curve.

There are two ways to increase resolution in SEC [56]. The first is by producing highly porous packings of controlled pore size. However, high porosity leads to mechanical instability. The second and more common practice is to use longer columns or multiple columns [57].

The primary requirement of the solvent used as the mobile phase is that it must dissolve the sample. The solvent must be compatible with the stationary phase to avoid swelling or shrinking of the pores [58]. The solvent must also be able to prevent analytes from adsorbing on to the stationary phase. The separation must be strictly due to size difference. The mobile phase must be flowed below the pressure limit of the column (1000 psi for SEC) [5].

SEC can be divided into two categories, namely gel filtration chromatography (GFC) and gel permeation chromatography (GPC). Gel filtration chromatography refers to separations using water as the mobile phase and is applicable to water-soluble polymers and proteins. Gel permeation chromatography is a term used for separations using organic mobile phases and has applications in polymer chemistry and petroleum analysis [45].

The types of GPC stationary phases include: polystyrene-divinylbenzene, poly-divinylbenzene; and dextran-based Sephadex columns [60-62]. These columns may have functional groups such as diol-modified silica, silica and poly (vinyl acetate) chosen to minimize analyte adsorption [63-65].

1.3.3 Detection

Eluent coming off the column is passed through a detection device that measures changes in some property of the eluent to detect the presence of analytes. This information is collected by a computer which records data and plots out a chromatogram. HPLC detectors used in petroleum analysis include spectroscopic detectors that identify substances through the spectrum emitted from them or absorbed by them (eg. infrared, fluorescence, and ultra-violet) [5]. Alternately, bulk property detectors which measure some bulk physical property of the eluent such as refractive index and evaporative light-scattering. Other detectors used include mass

spectroscopic, element-specific and electrochemical detectors. In the subsequent sections photodiode array detector (PDA) based on absorbance will be discussed, as this is the detector used throughout this thesis.

1.3.3.1 Absorbance Detector

Figure 1.10 is a schematic of an absorbance detector, where an eluent coming off the column is exposed to monochromatic light with radiant power P_o. The light passes through the sample and the radiant energy P of the emerging light is measured.

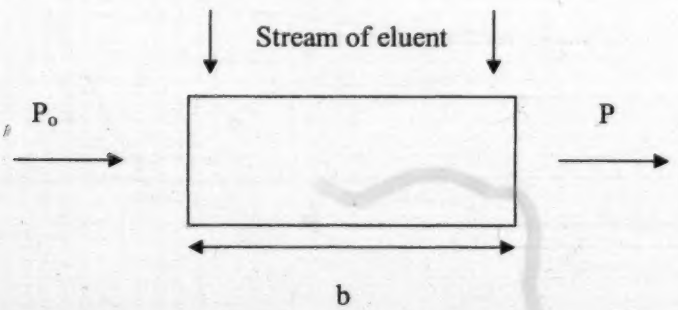


Figure 1.10 Absorbance detector where P_o is the radiant power entering into the flow cell, P is the radiant power coming out, and b is the path length.

The transmittance through the sample is defined as:

$$T = \frac{P}{P_o} \qquad (1.18)$$

If no energy is absorbed then $P = P_o$ and $T = 1$ or 100%. Transmittance and absorbance are related by:

$$\log T = -A \qquad (1.19)$$

Absorbance is directly proportional to the analyte concentration according to the Beer-Lambert Law:

$$A = \varepsilon bc \qquad (1.20)$$

where A is the absorbance (unitless, sometimes quoted in absorbance units, au), and ε is the molar absorptivity or extinction coefficient, a characteristic of a substance indicating how much light is absorbed at a particular wavelength (M^{-1} cm^{-1}). The path length, b, is measured in centimeters (cm), and c is the concentration (molarity, moles $Litre^{-1}$). Beer's law is applicable to dilute solutions only ($c \leq 0.01$ M). The linear relationship between absorption and concentration disappears at higher concentrations.

The absorption of ultraviolet-visible (UV-Vis) photons causes valence electrons in the analyte molecules to be promoted to higher energy orbitals (Figure 1.11), causing the analyte molecule to go from an initial energy state (the ground state) to another, higher energy state (an excited state). In this process, photons of light are absorbed by the analyte molecules, reducing the radiant power of the light emerging from the flow cell.

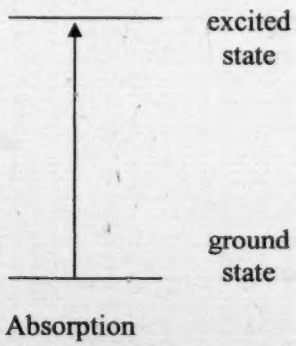


Figure 1.11 Energy level diagram. Adapted from reference [5,24].

One type of absorption detector light source is UV-Vis spectroscopy. UV-Vis spectroscopy uses light in the visible, near ultraviolet and near infrared ranges (200 - 800 nm). The UV-Vis absorption detector is unable to detect poor UV absorbants such as saturated hydrocarbons, carbohydrates and aliphatic polymers. Gas oil resins contain aromatics and polars, and may require gradient elution to perform the HPLC separation. UV-Vis detector is well suited for gradient elution because it is not sensitive to changes in the composition of the mobile phase. The UV-Vis absorption detector is excellent for aromatics and polars which absorb in the UV-Vis range [5,24]. Therefore UV-Vis spectroscopy was used in this thesis.

1.3.3.2 Photodiode Array Detector

The UV-Vis absorption spectrometer used in this thesis is equipped with a photodiode array (PDA) detector. PDA is composed of individual photosensitive silicon photodiodes with reverse-biased *pn* junctions. The photodiodes are part of an integrated circuit formed on a single silicon chip (Figure 1.12). Incident light creates charges in both the p and n regions. Electron-hole pairs are generated along the p-type bars and the n-regions [24,66]. The integrated circuit also contains a storage capacitor and switch for each diode (not shown here) [66]. The electron-hole pairs create a current that partially discharges the capacitor in the circuit. The resultant charge current required to re-charge the capacitor is proportional to the light radiant intensity. With PDA it is now possible to record the entire UV-Vis spectrum of the eluent in real time. Alternately, chromatograms can be recorded at multiple wavelengths simultaneously, enabling optimal detection of analytes even if their λ_{max} differ.

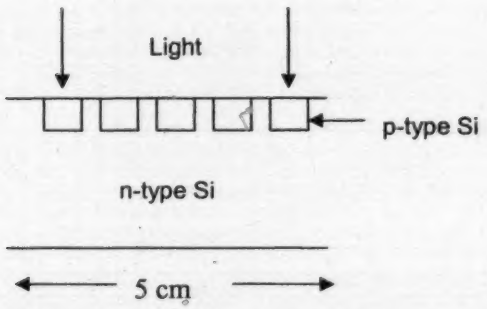


Figure 1.12 A photodiode array, composed of n-type silica and p-type silica. Not drawn to scale. The total length in this example is 5 cm. Adapted from reference [24].

The key to PDA detection is that white light (i.e. light of all wavelengths) passes through the sample (Figure 1.13), then through a polychromator, which disperses it into its individual wavelengths, and is finally detected by the photodiode array. A different band of wavelengths strikes each diode in the array. Conventional spectrophotometers scan through a spectrum one wavelength at a time. The PDA acquires the full spectrum simultaneously in seconds [24].

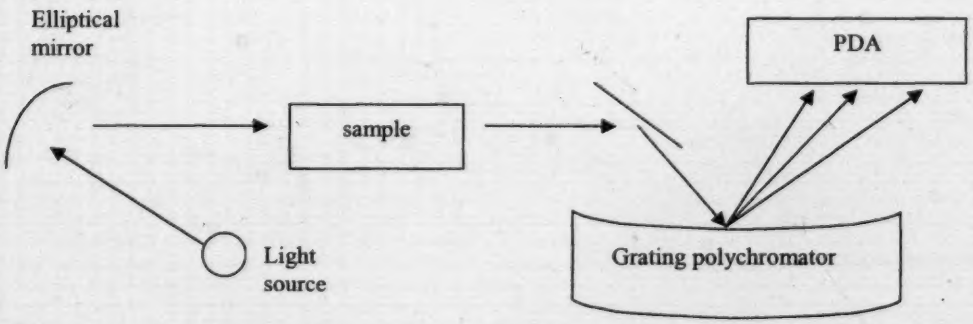


Figure 1.13 Photodiode array detector components. Adapted from reference [24].

1.3.4 Columns

1.3.4.1 Styrene-divinylbenzene copolymers

In 1934, the first styrene-divinylbenzene copolymers were developed by Staudinger and Heuer [67], for use as an ion exchange resin. They prepared the material by cross-linking co-polymerization of styrene with a small amount of divinylbenzene in the absence of solvent [38]. When dry, this copolymer is transparent and impermeable even to gas. When wet, it swells, revealing its hidden porosity. Swelling is an essential condition of the copolymer in order for it to become accessible to solutes. Applications of this first-generation copolymer are limited to solvents that can make it swell, such as toluene and ethylene dichloride.

In 1952, a second-generation styrene copolymer with large, permanent pores was developed by McBurney [68]. This second-generation was made by combining a solution of homogenous styrene with divinylbenzene and a porogen [38]. A porogen is a solvent that is miscible with the monomers, but its miscibility decreases as the chains of styrene copolymer grow. This solution then undergoes micro-phase separation followed by evaporation. The evaporation allows voids in copolymer structure to form, giving rise to interconnected macropores (> 200 Å) [38]. This second-generation styrene-divinylbenzene copolymer is opaque, with a total pore volume of 1 mL g^{-1} and a surface area of 20 – 300 m^2 g^{-1}. Unlike the first-generation copolymer, the second generation is not limited to toluene or ethylene dichloride [69].

Today there are many companies that offer styrene-divinylbenzene chromatography columns, including Waters, Shodex, Showa Denko and Tosoh, and each have their own formula [58]. Analyses of fossil fuels using styrene-divinylbenzene copolymer stationary phases are carried out with various pore sizes (50 to 10,000 Å) and with particle sizes in the 5 - 30 μm range [5,55]. In this thesis, model compounds representing components in gas oil resins are chromatographically separated using a Waters HR0.5 styrene-divinylbenzene column.

1.3.4.2 Hypercrosslinked polystyrene

Hypercrosslinked polystyrene is a third generation styrene stationary phase for GPC. The hypercrosslinked polystyrene consists of long polystyrene chains formed by introducing methylene (-CH_2-) bridges between phenyl groups of polystyrene in the presence of ethylene dichloride as the porogen[18] (Figure 1.14) [38]. Micropores of 20 – 50 Å diameter are formed [54]. These micropores are flexible and require over 40% crosslinking to be sufficiently rigid [69]. Increased rigidity allow hypercrosslinked polystyrene to be used at conventional HPLC pressures of 2000 –3000 psi, compared to <1000 psi with conventional styrene-divinylbenzene copolymer columns [52,57].

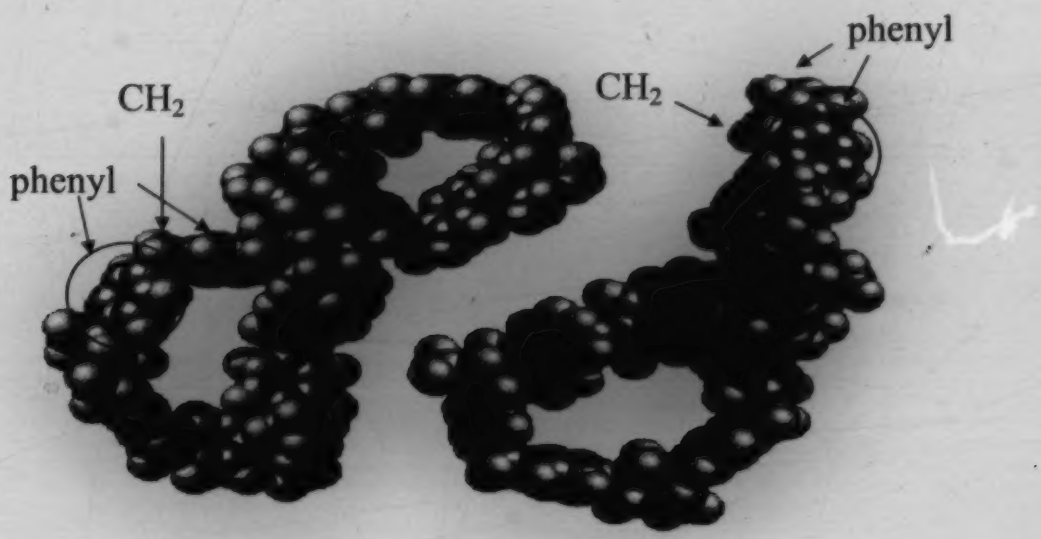


Figure 1.14 Structure of hypercrosslinked polystyrene, showing the -CH_2- bridges between each phenyl groups [38]. Reprinted with permission from Elsevier.

In addition, hypercrosslinked polystyrene can withstand temperatures up to 200 – 220°C [52]. This stationary phase is compatible with both hydrophobic solvents such as hexane, and chloroform, and with hydrophilic and polar solvents such as acetonitrile, methanol and water [54]. One limitation of this stationary phase is that a

[18] Porogen: Porogen is an additive to influence specific pore volume and pore size distribution.

given hypercrosslinked polystyrene column must be limited to only one mode of operation (either reverse-phase or quasi-normal); otherwise the beads may deform and separation efficiency will be lost (Section 1.3.1) [54]. Also, this column packing collapses if the solvent is allowed to evaporate [69].

Hypercrosslinked polystyrene is neutral with no polar groups, is usable over a pH range of 2 – 14, and is compatible with polar and non-polar organic solvents as well as water [54,69,71]. The phenyl groups in hypercrosslinked polystyrene participate in π - π interactions (Section 1.3.2.5) [69,71]. Hypercrosslinked polystyrene also has a high surface area (1000 – 1500 $m^2 g^{-1}$), which is larger than silica (100 – 600 $m^2 g^{-1}$) [72] or the second generation polystyrene-divinylbenzene copolymers (20 – 300 $m^2 g^{-1}$) [68].

Hypercrosslinked polystyrene has been developed further by Davankov and Tsyurupa [70-71], who have prepared Chromalite 5 HGN, a hypercrosslinked polystyrene gel, now commercially available from Purolite [54]. Chromalite 5 HGN is a 5 μm monosized, hypercrosslinked (H), gel-type (G), and non-activated (N) stationary phase. Chromalite 5 HGN has been used in adsorption preparative chromatography, size exclusion chromatography of polystyrene (Section 1.3.2.6) [54], and solid phase extraction[19] [69], but has yet to find wide application as an HPLC stationary phase [69,73-75]. In this thesis, Chromalite 5HGN is used as an HPLC stationary phase in quasi-normal phase mode.

In this thesis, three HPLC columns were evaluated using model compounds. In Chapter 2, gel permeation chromatography was investigated as a means of separating a series of aromatic compounds from naphthalene to pentacene, with limited success. In Chapter 3, the separations of model compounds by normal phase liquid chromatography using silica-based amino-cyano column and quasi-normal phase chromatography with a hypercrosslinked polystyrene stationary phase were compared. In Chapter 4, gas oil resins from different sources were analyzed using model compounds to determine whether separation of gas oil resins was achieved. Finally, Chapter 5 outlined future goals for the project.

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[19] Solid phase extraction: Solid phase extraction (SPE) is based on the preferential affinity of desired or undesired solutes for the solid phase.

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Chapter 2: Determining resolution capability of aromatic model compounds on a gel permeation chromatography

2.1 Introduction

In the 1960s, J.C. Moore of the Dow Chemical Company pioneered gel permeation chromatography (GPC) [1]. Separation is based strictly on the size and shape of the analyte molecule, and the average pore size and packing geometry of the stationary phase [2]. Large molecules elute first, followed by smaller molecules. GPC is a standardized method in the plastics industry for determining the molecular weight distribution of polymers [3-5], and is also used for simplification of matrices in foods and pharmaceutical preparation [6]. In this chapter, GPC for the separation of organics in petroleum is explored.

GPC is used in the petroleum industry to assess process quality and suitability, to monitor changes during chemical processing, and to evaluate fractions obtained from distillation [7]. Typically, the number-average molecular weight (M_n) and the weight-average molecular weight (M_w) of crude oil and petroleum products are reported based on polystyrene standards. These numbers carry a high degree of uncertainty, such that only the differences in elution profile are comparable but not the absolute molecular weight values [7]. Early applications include fingerprinting of crude oils [8-14] and analyses of asphalt [15-17]. Molecular weight distribution studies have also been done on asphaltenes and resins [18-20], but aromatic model compounds have not previously been analysed. The goal of this thesis is to separate gas oil resins into different components. In this chapter, polyaromatics ranging from naphthalene to pentacene known to be in gas oil resins [21] will be resolved using a high resolution GPC column.

In general, when GPC is applied to small (< 2000 MW) organic molecules, a 10% size difference must be present in order to observe separation [2,6]. Gas oil resins contain polyaromatic compounds ranging from naphthalene to pentacene, with molecular weights (MW) in the range 128 – 278 g mol^{-1} [21]. There is a 50 g mol^{-1} MW difference between each of the compounds, so separation by GPC can be expected. The target is to achieve baseline resolution for each peak ($R_s \geq 1.5$) and to

achieve an ideal GPC separation (characterized by a linear fit of log [MW] vs. elution time with correlation coefficient $R^2 \geq 0.99$). Variables that will be explored are the column, mobile phase, and temperature.

The Waters Styragel HR0.5 (high resolution) column is designed specifically for the separation of low-to-mid molecular weight samples (i.e., 100 – 1000 g mol^{-1}) [22] and, thus, is used here. Toluene and chloroform are two solvents commonly used for gas oils [23] and are effective at solubilizing aromatics possessing up to five rings. Thus, these solvents will be investigated here. Increased temperature will enhance solubility and improve mass transfer, and so will also be studied.

2.2 Experimental

2.2.1 Reagents

All solvents were HPLC grade, purchased from Fisher (Ottawa, ON, Canada) and pre-filtered using 0.5 μm filters. The samples were pre-filtered using a 0.20 μm polytetrafluoroethylene (PTFE)-fluorophore membrane from Millex (Columbus, Ohio, USA).

All aromatic model compounds were from Aldrich (Oakville, ON, Canada) and were prepared at 1 – 30 parts-per-thousand (ppt) concentrations (weight/weight). The purities of naphthalene, anthracene and tetracene were 99%, 99%, and 98%, respectively; the purity of pentacene was not specified (Figure 1.3). Polystyrene standards were TSK Standard Polystyrene from Tosoh Corporation (Grove City, OH, USA), containing polystyrene ranging in MW from 266 to 2980 g mol^{-1}, and were prepared at 4-30 wt%. The polystyrene standards came either as a single compound or a mixture. For those that contained a mixture, the reference chromatogram from the manufacturer was used to identify the MW of each peak.

2.2.2 Apparatus

The HPLC system consisted of a Beckman 125 Pump (Mississauga, ON), a Beckman 166 UV-Visible detector (300-700nm), a Rheodyne 7725i (Rohnert Park, CA) injector fit with a 5 μL loop, an Eppendorf CH-30 column heater (Mississauga, ON), and a Waters Styragel HR0.5 column (Waters Canada, Mississauga, ON). Wide bore (0.02-inch or 0.5 mm ID) tubing was used prior to the injector and narrow (0.009-inch or 0.25 mm ID) tubing was used after the injector to minimize extra-column band broadening and achieve the recommended <75 μL peak broadening (at 4.4% peak height) [22].

The Waters Styragel HR0.5 (high resolution) column was a 4.6 mm ID x 300 mm column designed specifically for the analysis of low-to-mid molecular weight samples [6]. The column was packed with rigid, spherical 5 μm styrene-divinylbenzene particles possessing 50 Å pores. The column has an effective MW range of 100 – 1000 g mol^{-1} over a 5 mL elution window. These styragel columns are available packed in tetrahydrofuran, dimethylformamide or toluene mobile phases.

When choosing the appropriate mobile phase several parameters must be considered. First, toluene and chloroform are the two solvents commonly used for liquid chromatography of gas oil [23]. Second, changing the solvent used with a column may pose problems of solubility and compatibility with the previous solvent and the stationary phase [2]. In addition, frequent solvent switches will accelerate column deterioration and loss of resolution [22]. Taking these factors into consideration, we chose the toluene-packed Waters HR0.5. The column and standards were first studied using toluene, and then using chloroform.

2.2.3 Method

The mobile phase (toluene or chloroform) was pumped through the column at a flow rate of 0.3 mL min^{-1}, resulting in an operating pressure of 220 psi. The styrene-divinylbenzene column must be kept below 500 psi because the chromatographic bed can easily compact under pressure [22]. To avoid column compaction the flow rate

was gradually increased at 0.1 mL min^{-1} increments at 30 second intervals until the final flow rate of 0.3 mL min^{-1} was reached [22]. GPC of the polyaromatic standards was first studied using toluene. To convert the Waters HR0.5 column to chloroform, the column was flushed with chloroform at 0.1 mL min^{-1} for 8 hours.

The Waters HR0.5 column was kept at a constant temperature of 35°C, 60°C or 80°C using an Eppendorf CH-30 column heater that had a ± 1°C absolute accuracy. The manufacturer recommends changing the temperature no faster than 10°C every 30 minutes with a 0.1 mL min^{-1} solvent flow [22]. In this work the temperature was changed at ≤ 10°C every 10 minutes, with no observable loss in efficiency based on the quality control standard.

Samples were injected in 5 μL volumes so as not to overload the column. The UV-Vis (ultraviolet-visible) absorbance detector was set to monitor each compound's strongest absorbing wavelength, or as indicated. Naphthalene was observed at 275 nm, anthracene at 375 nm, tetracene at 475 nm and pentacene at 360 nm. The data acquisition rate was set to 5 Hz, with a 0.5 second response time.

Anthracene (0.0266 wt% in toluene or chloroform) was used as the quality control sample, using a flow rate of 0.3 mL min^{-1} and monitoring at 340 nm. For this sample a retention volume deviation of 2.5% was observed (over 6 injections for each mobile phase system), and the chromatographic efficiency (N), calculated using the peak width at baseline, was 13,000 plates (Equation 1.13).

2.3 Results and Discussion

2.3.1 Toluene

2.3.1.1 Polystyrene and aromatic model compounds in toluene at 35°C

The Waters Styragel HR0.5 column has an effective MW range of 100 – 1000 g mol^{-1} [6]. To confirm this, polystyrene standards were run at 0.3 mL min^{-1}, 340 nm and 35°C in toluene. Figure 2.1 shows the elution order of the 266 – 870 g mol^{-1} polystyrene standards. The 2980 g mol^{-1} standard is totally excluded from

the stationary phase's pores and so elutes at 2.45 mL. The data shows the expected [22] linear relationship between log [MW] and retention volume (V in mL) (correlation coefficient R^2 = 0.996). This indicates complete solubility of 266 – 870 g mol^{-1} polystyrene in toluene and complete elution of the samples from the column during each run.

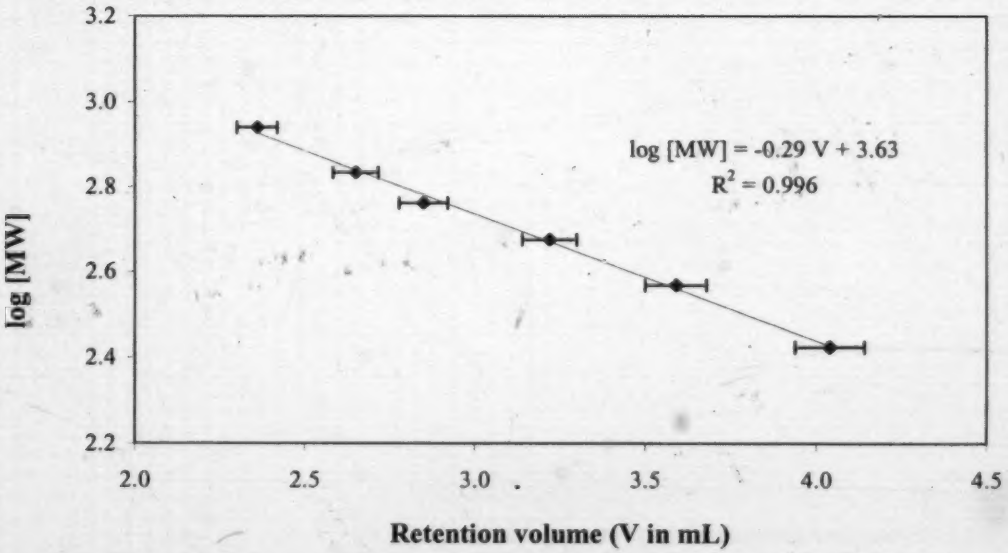

Figure 2.1 Retention of polystyrene standards on a 4.6 mm ID x 300 mm Waters HR0.5 column, monitored by absorbance at 340 nm. The separation was performed using a toluene mobile phase at 35°C with a flow rate of 0.3 mL min^{-1}. The line is a linear regression fit with the indicated equation and correlation coefficient; the 2.5% error bars are based on the retention volume deviation observed for the quality control standard.

Aromatic model compounds ranging from naphthalene (MW 128 g mol^{-1}) to pentacene (MW 278 g mol^{-1}) were separated under the same conditions as the polystyrene standards, except absorption wavelength was modified as indicated in Section 2.2.3. The resolutions were calculated based on separate injections of each aromatic model compound. The value for each is computed relative to the next lightest compound. All four compounds elute over a narrow range of 0.25 mL. In addition, none of the peaks are baseline resolved ($R_s \geq 1.5$) (Table 2.1). In general, increasing

the flow rate would decrease the theoretical plate height, in turn improving resolution (Section 1.3.1) [24]. However, high flow rates are not possible in GPC due to the 500 psi pressure limit of styrene-divinylbenzene columns [22]. Therefore, the efficiency of separation could not be studied as a function of flow rate.

Table 2.1 Resolution of aromatic model compounds on a 4.6 mm ID x 300 mm Waters HR0.5 column using a toluene mobile phase at 35°C and a flow rate of 0.3 mL min^{-1}.

Sample	Molecular Weight ($g\ mol^{-1}$)	Retention Volume (mL)	W_B (mL)	Resolution
naphthalene	128	4.14	0.16	
anthracene	178	4.11	0.17	0.2
tetracene	228	3.91	0.17	1.2
pentacene	278	3.89	0.53	0.1

The resolution value listed for each compound is computed relative to the next lightest compound and based on separate injections of each aromatic model compound.

To determine if an accurate and optimum separation was attained, a plot of log MW vs elution volume was made (Section 1.3.2.6 and Figure 1.9). The filled diamonds in Figure 2.2 show the observed retention behavior for the aromatic model compounds. A linear regression fit through the aromatic retention volume data gave a correlation coefficient, R^2, of only 0.78. The non-linear fit indicate that GPC using toluene at 35°C provides inadequate separation of the model aromatic compounds, and, given the narrow elution window, would provide essentially no separation of the aromatic components of gas oil resins.

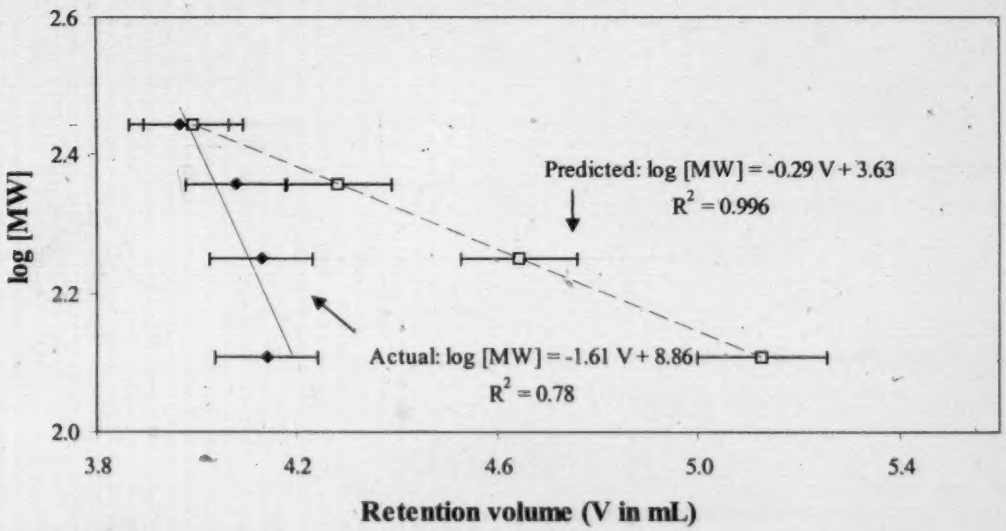

Figure 2.2 Retention of aromatic model compounds on a 4.6 mm ID x 300 mm Waters HR0.5 column, monitored by absorbance at the wavelengths indicated in Section 2.2.3. GPC was performed using a toluene mobile phase at 35°C and a flow rate of 0.3 mL min^{-1}.

Experimental (filled diamonds) and predicted (open squares) data points are shown. The line is a linear regression fit with the indicated equation; the 2.5% error bars are based on retention volume deviation observed for a quality control sample.

An effort was made to determine why GPC using toluene at 35°C failed. One possible explanation for this non-ideality is the presence of adsorption onto the column. To test whether this is the case, the polystyrene linear equation shown in Figure 2.1 was used to predict the retention volumes of the aromatic model compounds (MW 128 – 278 g mol^{-1}). The open squares in Figure 2.2 show retention volumes predicted in this way. If there were adsorption onto the column, the aromatics would have eluted later than predicted, but instead the opposite is true, ruling out this possibility.

Another possibility is that the aromatics are aggregating and moving through the column as larger clusters of molecules, thus eluting earlier than predicted. Indeed, aggregation has previously been observed for a larger and more polar analog of gas oil

resins, namely asphaltenes. Using computer modeling, Murgich and Strausz [25] found resins aggregate with asphaltenes in the presence of toluene. In addition, Donaldson and co-workers [26] had difficulty keeping asphaltenes in toluene solution during GPC analyses of asphalt. They observed a smaller retention volume for asphaltenes in toluene relative to asphaltenes in tetrahydrofuran. However, asphaltenes and resins contain polar species (acids, bases and heteroaromatics) which are believed to cause the aggregation [21]. The model compounds being used here are non-polar, that is, pure linear aromatics are being studied here, which are not expected to aggregate.

A third hypothesis is that the aromatics elute earlier than expected because the pore size has changed. For this column, Waters reports an effective molecular range of 100 – 1000 g mol^{-1} and an average pore size of 50 Å [6,22], but the pore size varies depending on the solvent [27,28]. For example, Hall and co-workers [27] found that toluene causes the styrene-divinylbenzene beads to swell by a factor of 1.2 – 1.6 times greater than in their dry state. Swelling causes expansion of the polymer structure thus increasing average pore size [27]. The pore size is one of the factors that determines the effective molecular weight range (Section 1.3.2.6) [27,28]. The predicted retention volumes were calculated assuming that the trend observed for the polystyrene standards (Figure 2.1, MW 266 – 870 g mol^{-1}) would continue for aromatics (Figure 2.2, 128 – 278 g mol^{-1}). Perhaps in toluene, naphthalene and anthracene deviate from the linear trend line indicating total permeation into the pores (Figure 1.9).

2.3.1.2 Polystyrene and aromatic model compounds in toluene at 80°C

According to Van Deemter's rate theory [24], resistance to mass transfer is the dominant factor in peak broadening. Increasing rate of mass transfer of analyte molecules in and out of the stationary phase at higher temperatures would reduce peak broadening, thus increasing resolution (Section 1.3.1). In the hope of improving resolution by narrowing peak width, the experiments described in the previous section were repeated at a higher temperature.

To avoid having to use a back pressure regulator the temperature was set below the boiling point of toluene (110.6°C) [29]. Polystyrene standards and aromatic model compounds were separated under the same conditions as in Section 2.3.1.1, except the column temperature was raised to 80°C. The filled diamonds in Figure 2.3 show the observed retention behavior for the aromatic model compounds at 80°C. The elution range narrowed to 0.20 mL and gave a non-linear MW calibration (log [MW] vs. retention volume, correlation coefficient $R^2 = 0.72$).

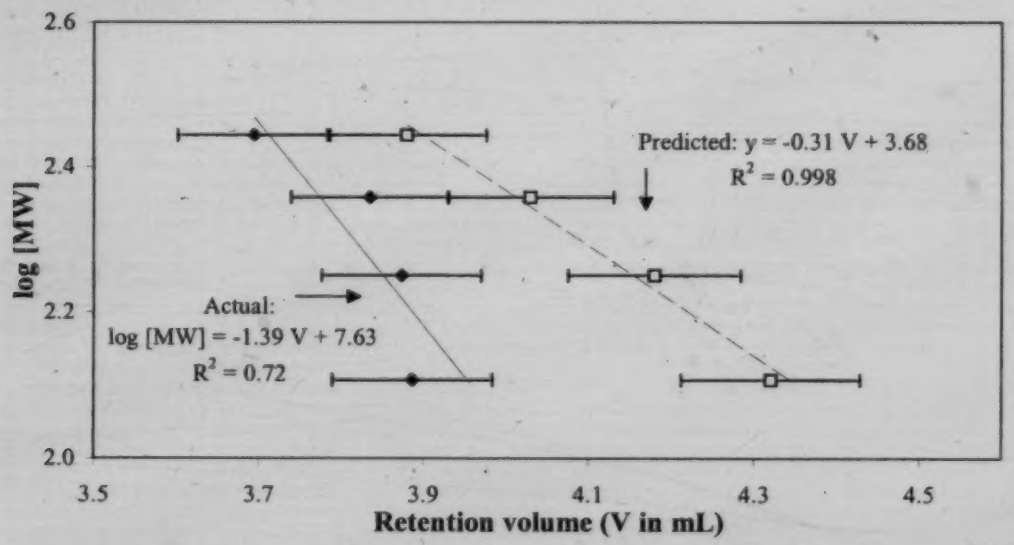

Figure 2.3 Retention of aromatic model compounds on a 4.6 mm ID x 300 mm Waters HR0.5 column, monitored by absorbance at the wavelengths indicated in Section 2.2.3. GPC was performed using a toluene mobile phase at 80°C and a flow rate of 0.3 mL min^{-1}.

Experimental (filled diamonds) and predicted (open squares) data points are shown. The line is a linear regression fit with the indicated equation; the 2.5% error bars are based on retention volume deviation observed for a quality control sample.

According to the mass transfer argument above, at this higher temperature peak widths should have decreased for all analytes (Table 2.2). A possible explanation could be that the temperature needed to be increased further to overcome the resistance to mass transfer. However, none of the aromatics were baseline resolved ($R_s \geq 1.5$).

Retention volumes for the aromatic model compounds at 80°C have been predicted by extrapolating data from polystyrene standards run under the same conditions (open squares in Figure 2.3). The aromatics are still eluting earlier than expected. Overall, toluene at 80°C failed to resolve and linearly elute the series of aromatic model compounds, therefore another mobile phase must be considered.

Table 2.2 Resolution of aromatic model compounds on a 4.6 mm ID x 300 mm Waters HR0.5 column using a toluene mobile phase at 80°C and a flow rate of 0.3 mL min^{-1}.

Sample	Molecular Weight ($g\ mol^{-1}$)	Retention Volume (mL)	W_B (mL)	Resolution
naphthalene	128	3.89	0.17	
anthracene	178	3.87	0.15	0.1
tetracene	228	3.83	0.17	0.3
pentacene	278	3.69	0.20	0.8

The resolution value listed for each compound is computed relative to the next lightest compound and based on separate injections of each aromatic model compound.

2.3.2 Chloroform

2.3.2.1 Polystyrene and aromatic model compounds in chloroform at 35°C

In an attempt to improve upon the separation results, a more polar mobile phase, chloroform, was chosen. The same Waters HR0.5 column, which was previously packed using toluene, was changed to chloroform (see Section 2.2.3). Polystyrene standards and aromatic model compounds were run at 35°C in chloroform with a flow rate of 0.3 mL min^{-1}. Due to the UV cutoff of chloroform (245 nm) [29], the polystyrene standards were monitored at 280 nm, while the aromatics were observed at the absorption wavelengths indicated in Section 2.2.3.

The retention volumes of the aromatic model compounds in chloroform are shown as filled diamonds in Figure 2.4. The compounds eluted over a wider range of

retention volumes (0.76 mL) compared to the results in toluene (0.25 mL, Figure 2.2). Going from toluene to chloroform improved the linear correlation coefficient of the log [MW] vs. retention volume plot from $R^2 = 0.78$ to $R^2 = 0.83$. The resolution of all the peaks increased (Table 2.3 vs. Table 2.1). However, even with these improvements only anthracene and tetracene were baseline resolved (Table 2.3, Resolution = 4.7).

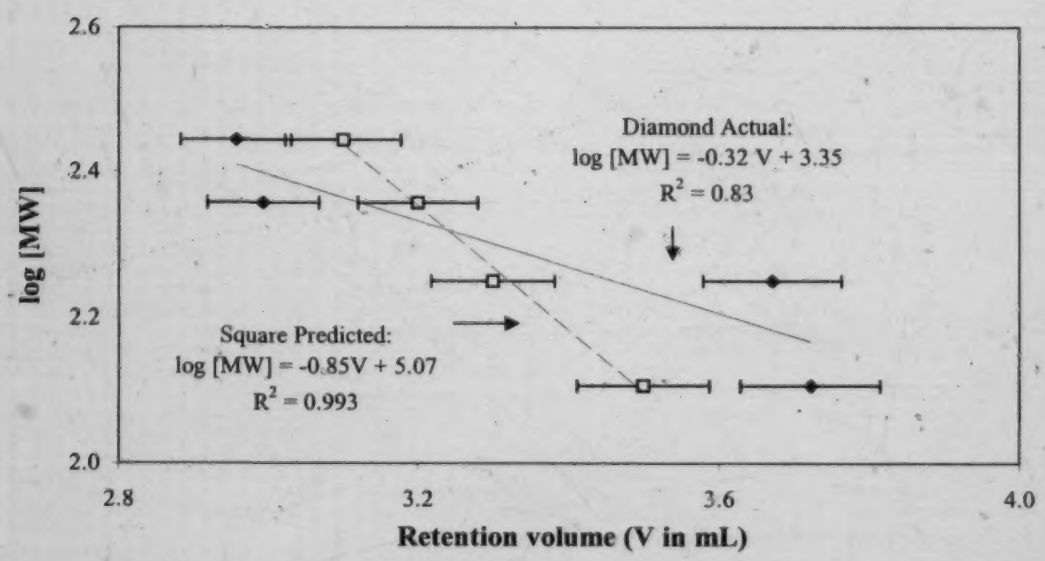

Figure 2.4 Retention of aromatic model compounds on a 4.6 mm ID x 300 mm Waters HR0.5 column, monitored by absorbance at the wavelengths indicated in Section 2.2.3. GPC was performed using a chloroform mobile phase at 35°C and a flow rate of 0.3 mL min^{-1}.

Experimental (filled diamonds) and predicted (open squares) data points are shown. The line is a linear regression fit with the indicated equation; the 2.5% error bars are based on retention volume deviation observed for a quality control sample.

As in previous sections, the predicted values were determined by extrapolating from polystyrene standard data obtained under the same experimental conditions as the aromatic model compounds. From Figure 2.2, the predicted elution range is from 3.1 – 3.5 or over 0.4 mL (open squares). The experimentally observed range of retention

volumes were from 2.96 to 3.72 mL, or 0.76 mL (filled diamonds). In fact, the aromatic model compounds eluted over a longer range than predicted, which indicates that chloroform was a better solvent for aromatics than for polystyrene. Since the aromatic compounds were no longer eluting earlier than expected, it appears that chloroform did not swell the styrene-divinylbenzene stationary phase as much as toluene did. This is consistent with the observations of Ogino and Sato [28] who used toluene to swell styrene-divinylbenzene and chloroform to measure the amount of swelling.

Table 2.3 Resolution of aromatic model compounds on a 4.6 mm ID x 300 mm Waters HR0.5 column using a chloroform mobile phase at 35°C and a flow rate of 0.3 mL min^{-1}.

Sample	Molecular Weight ($g\ mol^{-1}$)	Retention Volume (mL)	W_B (mL)	Resolution
naphthalene	128	3.72	0.14	
anthracene	178	3.67	0.15	0.4
tetracene	228	2.99	0.15	4.7
pentacene	278	2.96	0.13	0.2

The resolution value listed for each compound is computed relative to the next lightest compound and based on separate injections of each aromatic model compound.

2.3.2.2 Polystyrene and aromatic model compounds in chloroform at 50°C

In an attempt to obtain better resolution and a more linear log [MW] vs. retention volume profile for the series of aromatic model compounds, the experiment described in Section 2.3.2.1 was repeated at a higher temperature. To avoid having to use a back pressure regulator, the temperature was set below the boiling point of chloroform (62°C), to 50°C [29]. The linear correlation coefficient, R^2, increased from 0.83 to 0.87 in going from 35°C (Figure 2.4) to 50°C (Figure 2.5), which indicates a small improvement in linearity. However, for each aromatic model compound the

retention volumes overlap within the 2.5% error bars for 35°C and 50°C (Figure 2.5). There may not be a statistically significant difference between the two sets of data.

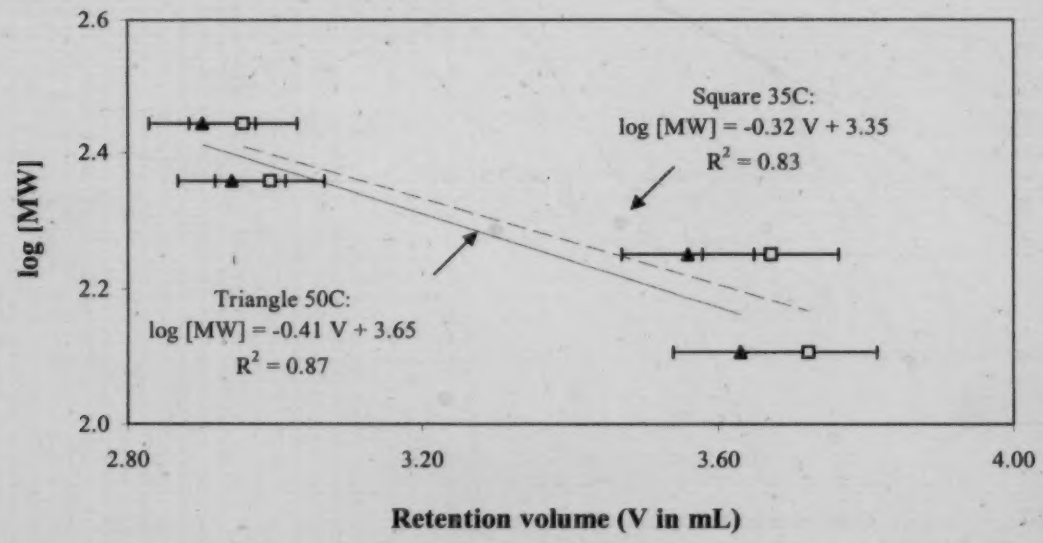

Figure 2.5 Retention of aromatic model compounds on a 4.6 mm ID x 300 mm Waters HR0.5 column, monitored by absorbance at the wavelengths indicated in Section 2.2.3. GPC was performed using a chloroform mobile phase at 35 °C (open squares) and 50°C (filled triangles) and a flow rate of 0.3 mL min^{-1}.

The lines are linear regression fits with the indicated equation; the 2.5% error bars are based on retention volume deviation observed for a quality control sample.

Table 2.4 Resolution of aromatic model compounds on a 4.6 mm ID x 300 mm Waters HR0.5 column using a chloroform mobile phase at 50°C and a flow rate of 0.3 mL min^{-1}.

Sample	Molecular Weight ($g\ mol^{-1}$)	Retention Volume (mL)	W_B (mL)	Resolution
naphthalene	128	3.63	0.17	
anthracene	178	3.56	0.15	0.4
tetracene	228	2.94	0.17	3.9
pentacene	278	2.90	0.20	0.2

The resolution value listed for each compound is computed relative to the next lightest compound and based on separate injections of each aromatic model compound.

A t-test was performed on the individual differences in retention volume observed for each aromatic model compound (Section 1.2, Equations 1.5 and 1.6) [30]. In this case d_{ave} is the average difference in retention volume between 35°C and 50°C, n is the number of pairs of data points (in this case 4). Recall that each data point in Figure 2.5 has a 2.5% retention volume deviation, thus there will be a maximum and minimum value for each data point. The values for 35°C were compared to the maximum values for 50°C as seen on Table 2.5. For 4 data points with 3 degrees of freedom the t_{table} = 3.182 [30]. The $t_{calculated}$ = 0.29 is less than the t_{table} = 3.182 (Table 2.5), therefore chloroform at 35°C and chloroform at 50°C are not statistically different. Going to 50°C does not result in a more linear elution order.

By going to higher temperature, the resolution of naphthalene and anthracene increased from 0.35 to 0.44. However, the critical peaks of tetracene and pentacene ($\Delta MW = 50\ g\ mol^{-1}$) were still not well-resolved (R_s = 0.2). All four compounds eluted over a range of 0.7 mL, and they were not all baseline resolved (Table 2.4). Therefore, using a chloroform mobile phase at 50°C will still not resolve the different aromatic components of gas oil resins.

Table 2.5 T-test of GPC performed using a chloroform mobile phase at 35 °C and 50°C.

Log (MW)	Retention volume at 35°C	Maximum retention volume at 50°C	Difference
2.11	3.72	3.72	0.00
2.25	3.67	3.65	-0.02
2.36	2.99	3.01	0.02
2.44	2.96	2.97	0.01
		Average Difference (d_{ave})	0.002
		Standard Deviation (s_d)	0.02
		$t_{calculated}$	0.29

2.4 Conclusion

In an ideal GPC experiment, analytes are separated according to differences in their molecular weight and effective size in solution, with large molecules eluting first and the smaller molecules eluting last (Section 1.3.2.6, Figure 1.8). The calibration curve (log [MW] vs. retention volume) should be linear (correlation coefficient $R^2 \geq 0.99$), allowing analyte molecular weights to be determined with accuracy and precision (Section 1.3.2.6, Figure 1.9). The goal of this chapter was to determine whether GPC can separate aromatic model compounds ($R_s \geq 1.5$), as a model for aromatic materials present in gas oil resins.

At 35°C, aromatics in toluene and chloroform gave non-linear fits with correlation coefficients of 0.78 and 0.85, respectively. Neither mobile phase gave an ideal GPC separation. At best, using a chloroform mobile phase at 50°C, the model compounds gave a linear correlation coefficient R^2=0.85 and a minimum resolution of 0.22. None of the conditions investigated provided baseline resolution ($R_s \geq 1.5$) for all peaks.

Based on the present results using chloroform as the mobile phase, 56 columns would be required to achieve baseline resolution of the critical peaks of tetracene and

pentacene (ΔMW = 50 g/mol) (R_s = 0.22 from Table 2.4). This would increase the cost of the columns to over $50,000, and the analysis time to over 12 hours per run.

Toluene and chloroform were studied here because they dissolve gas oil resins the best [31]. Multiple changes in the solvent used with a column may pose problems of solubility and compatibility with previous solvents and the stationary phase [2], deterioration of the column and loss of resolution [22]. Thus, another column would have to be purchased to study any other solvent systems (Chapter 5).

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Chapter 3: Determining resolution and elution order of aromatic model compounds on a silica-based amino-cyano column and a hypercrosslinked polystyrene stationary phase

3.1 Introduction

Existing standardized HPLC methods for analyzing petroleum products are limited to middle distillates (boiling point range 150 – 400°C) [1] containing only small quantities of nitrogen and sulphur (<1% each) [2,3]. The current methods use silica-based columns which may irreversibly retain nitrogen and sulphur compounds [4]. Therefore, there is a need to find another type of column that is suitable for gas oil resins.

Chromalite 5HGN is a neutral hypercrosslinked polystyrene stationary phase with 5 μm monodispersed beads [6,7]. The hypercrosslinked polystyrene has no polar groups, usable in the pH range 2 – 14 and compatible with both polar and non-polar organic solvents. Hypercrosslinked polystyrene would be more appropriate for gas oil resins because it is compatible with both hydrophobic solvents, such as hexane and chloroform, and with hydrophilic and polar solvents, such as acetonitrile, methanol and water. The mobile phase can be easily modified to ensure sulphur- and nitrogen-containing compounds elute completely from the column [6-11].

In this chapter hexane and chloroform are used to elute aromatic (1 – 5 aromatic rings) and sulphur- and nitrogen-containing model compounds. The elution order of selected aromatic model compounds is determined for both a silica-based amino-cyano column and a hypercrosslinked polystyrene column. The silica-based amino-cyano column is used in normal phase liquid chromatography mode (Section 1.3.2.1). The hypercrosslinked polystyrene is used in quasi-normal phase chromatography (QNLC) mode (Section 1.3.25). The stability of the columns will be determined based on the reproducibility of observed retention times. In the end, the best column for analyzing gas oil resins will be determined.

3.2 Experimental

3.2.1 Reagents

All solvents were HPLC grade, purchased from Fisher (Ottawa, Ontario, Canada), and were pre-filtered by the manufacturer using 0.5 μm filters. The samples were pre-filtered with 0.20 μm polytetrafluoroethylene (PTFE)-fluorophore membrane from InnoSep (Samutsakorn, Thailand). Helium (>99.99%) for sparging of the mobile phase (Section 1.2.1) was from BOC Gas (Mississauga, Ontario, Canada). No additional drying of the solvents was performed.

Aromatic model compounds were prepared at concentrations (weight/weight) of 0.05 – 20 parts-per-thousand (ppt) in either hexane or 80:20 (vol%) hexane:chloroform. Tables 3.1 and 3.2 below list the source and purity of each model compound. The sources were: Fisher (Ottawa, Ontario, Canada), Sigma-Aldrich (Oakville, Ontario, Canada), Fluka (Seelze, Germany), TCI America (Portland, Oregon, USA), Eastman Organic Chemicals (Kingsport, Tennessee, USA), Acros Organics (Morris Plains, New Jersey, USA), and Matheson, Coleman and Bell (Norwood, Ohio, USA).

Table 3.1 Characteristics of aromatic and non-aromatic model compounds.

Compound	Source	Purity	Boiling Point (°C) [12]	Absorption maxima (nm) [12-14]
NON-AROMATIC				
cyclohexane	Aldrich	99.9%	80.9	U
AROMATIC				
Phenyl-branched				
o-xylene	Aldrich	98%	144	U
9-methylanthracene	Aldrich	97%	196.7	252,365,385
Linear				
benzene	Aldrich	99%	80.1	178
naphthalene	Aldrich	99%	218	268,275,278
anthracene	Aldrich	99%	340	328,345,363
tetracene	Aldrich	98%	U	U
pentacene	Aldrich	U	525	303,351
Fused angled				
phenanthrene	Aldrich	98%	340	250,292
pyrene	Aldrich	98%	404	U
benzo(e)pyrene	Aldrich	99%	492	204,279,290, 319,335
Biphenyl linked				
biphenyl	Aldrich	99.5%	255	245
p-terphenyl	Aldrich	99%	383	205,278
p-quaterphenyl	Fluka	U	428	206,296
Dihydroaromatic				
tetralin	TCI America	U	207	197,213, 266,273
9,10- dihydroanthracene	Aldrich	97%	312	209,267,301
5,12-dihydroanthracene	Aldrich	U	U	U

U: unspecified

Absorption maxima: Peak absorption wavelengths in each compound's ultraviolet/visible spectrum

See Figure 1.3 for structures of aromatic compounds.

Table 3.2 Characteristics of aromatic sulphur- and nitrogen-containing model compounds.

Compound	Source	Purity	Boiling Point (°C) [12]	Absorption maxima (nm) [12-14]
Sulphur				
Thiol:				
2-naphthalenethiol	Eastman Organic Chemicals	U	286	240,270, 280,290
Sulfide:				
phenyldisulfide	Aldrich	99%	U	U
phenylsulfide	Aldrich	98%	296	U
Thiophene:				
dibenzothiophene	Aldrich	98%	332	237,288
Nitrogen				
Pyrrole:				
carbazole	Acros Organics	96%	355	235,253,296
Pyridine:				
quinoline	Matheson, Coleman and Bell	U	238	226,230,281
Amine:				
2-aminonaphthalene	Aldrich	U	306	236,269,278, 290,336
Nitrogen/Oxygen				
nitrobenzene	Acros Organics	> 95%	210.8	262

U: unspecified

Absorption maxima: Peak absorption wavelengths in each compound's ultraviolet/visible spectrum

See Figure 1.4 for structures of sulphur- and nitrogen-containing model compounds.

3.2.2 Apparatus

The HPLC system was composed of a Waters 600 controller and multisolvent delivery system (Waters, Milford, Massachusetts, USA and Waters Canada, Mississauga, Ontario, Canada), a Waters 717 autosampler, a Waters 996 multiwavelength photodiode array detector and a Waters 2414 refractive index detector. Data was collected with an acquisition rate of 1 Hz and a response time of 1 second using Empower Pro 2 software from Waters. The Partisil 5PAC (Whatman, Brentford, Middlesex, England) and Chromalite 5HGN (Purolite International Limited, Wales, United Kingdom and Bala Cynwyd, Pennsylvania, USA) columns were temperature controlled using an Eppendorf CH-30 column heater (Mississauga, Ontario, Canada). The hypercrosslinked polystyrene stationary phase was packed in-house in a 4.6 mm ID x 250 mm stainless steel column with a 0.5 μm Rheodyne (Rohnert Park, California, USA) frit at each end. Wide-bore (0.02 inch or 0.5 mm ID) tubing was used prior to the injector and narrow-bore (0.009 inch or 0.25 mm ID) tubing was used after the injector.

Hypercrosslinked polystyrene is not available as a pre-packed column so a column packing method was devised. Polystyrene columns are typically used for size exclusion chromatography (SEC). Hypercrosslinked polystyrene is a third generation polystyrene material (Section 1.3.4.2), so the general method for SEC column preparation should be applicable. According to Wu and associates [15,16] it is important to obtain good distribution of beads to ensure a homogenous and stable stationary phase, and check separation efficiency and peak shape. Based on guidelines published by Wu *et al.* [15,16] and on the solvents and apparatus available in the laboratory, the following steps were taken to pack the hypercrosslinked polystyrene column.

The stationary phase beads were suspended in chloroform to create a slurry [16]. Chloroform was chosen because of its high density (1.50 g mL^{-1}), which enabled hypercrosslinked polystyrene beads to float. In addition, chloroform prepares the column for quasi-normal phase chromatography (Section 1.3.2.5) [6-8,11]. To prevent particle aggregation and trapped air, the particle-to-solvent ratio must be less than 70%

[15,16], so 10 g beads were sonicated with 200 mL chloroform. The mixture was poured into the reservoir (Figure 3.1, volume 170 mL, length 8.8 cm) with mechanical stirring using a magnetic bar. Once the slurry formed, the stir bar was removed to prevent damage to the beads.

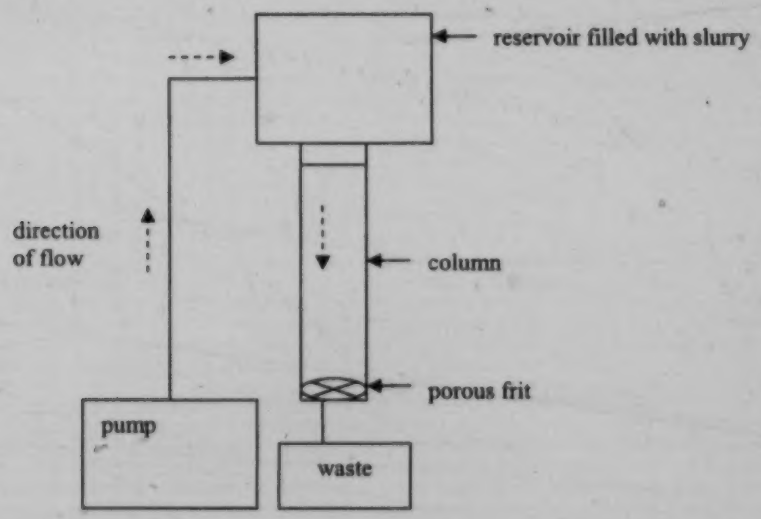

Figure 3.1 Apparatus for packing hypercrosslinked polystyrene column.

To ensure a homogenous and stable stationary phase, a constant flow velocity or pressure must be used to create a uniform drag force, thus preventing agglomeration of the particles at the bottom of the column [15,16] (Figure 3.1). A higher pressure than the expected operational pressure was used for packing, to ensure that the column could withstand different experimental conditions such as temperature or solvent variations. The typical packing pressure for semi-rigid polymer gels is 2000 – 4000 psi [15,16]. It was therefore decided to pack the hypercrosslinked polystyrene column at constant pressure of 3000 psi. The reservoir was attached to a high-pressure pump to force the slurry into an empty Whatman (Brentford, Middlesex, England) column (4.6 mm ID x 250 mm). The outlet end of the column had a 0.5 μm porous frit that allowed solvent to pass through while retaining the packing. Once the column was full, it was detached from the apparatus and another 0.5 μm porous frit was placed at the inlet end of the column.

The following procedure was used to test the separation efficiency and peak shape. A sample of nitrobenzene (0.5 ppt in 70:20:10 (vol%) hexane:dichloromethane:2-propanol) was injected onto the column (5 μL injection) and eluted at 60°C using a 70:20:10 (vol%) hexane:dichloromethane:2-propanol mobile phase with a flow rate of 1 mL min^{-1}, with elution monitored by absorbance at 254 nm. The separation efficiency, N, and USP tailing factor, T_f, were then calculated from the observed retention time and peak width using equations (1.14) and (1.15). The hypercrosslinked polystyrene column packed using the above procedure gave N = 5,000 plates and T_f = 1.5. For comparison, Purolite [11] quoted an N = 6,250 plates for the 250 mm hypercrosslinked polystyrene column described here. As the observed separation efficiency and peak shape were deemed acceptable, no further packing optimization steps were taken.

3.2.3 Method

All HPLC experiments were performed using either hexane or 80:20 (vol%) hexane:chloroform mobile phase, with a flow rate of 1 mL min^{-1}, oven temperature at 35°C regulated with ±1°C absolute accuracy, and an operating pressure of 580 psi. All solvents were sparged with helium for 15 minutes at 100 mL min^{-1} before use, and then sparged at 50 mL min^{-1} for the duration of the experiment. The Partisil 5PAC silica-based amino-cyano column (Section 1.3.2.1) and the Chromalite 5HGN hypercrosslinked polystyrene column (Sections 1.3.2.5 and 1.3.4.2) being compared were the same size (4.6 mm ID x 250 mm), and both stationary phases had the same particle size (5 μm). For experiments using either mobile phase, both columns were equilibrated with the mobile phase at 1 mL min^{-1} for 60 minutes (totaling 15 times the column volume) at the beginning of each day before making injections.

For experiments using a hexane mobile phase, chromatograms were recorded using a refractive index detector. For the Partisil 5PAC column, sample injections of 10 μL were sufficient to observe elution of all peaks. For the hypercrosslinked polystyrene column, the peaks were broader so the injection volume was increased to 50 μL. The retention time of an un-retained solute (t_o) can be approximated using

Equation (1.11), where t_o is 2.6 minutes for a 4.6 mm ID x 250 mm column at a flow rate of 1 mL min^{-1} [17]. The retention time of heptane (3.0 minutes for both columns) was taken as the t_o.

For experiments using an 80:20 (vol%) hexane:chloroform mobile phase a photodiode array (PDA) detector was used because the PDA is more sensitive to aromatics than the refractive index detector (Section 1.3.3.2). An injection volume of 10 µL was sufficient for both columns. This injection volume did not overload the columns and kept the PDA detector's signal within its linear response range of < 2.0 au (Equation 1.20) [18]. Chromatograms were recorded over a wavelength range of 210 – 400 nm, in which all the aromatic model compounds absorb (Tables 3.1 and 3.2). Reference spectra were used to confirm eluent identity [12-14]. Heptane is a poor UV absorbant (Section 1.3.3.2) therefore the un-retained solute was changed to 1-phenyl octane with a t_o of 3.3 minutes (Equation 1.10).

A quality control sample was analysed periodically to check column stability. Anthracene, 82 parts-per-million (ppm by weight) in 80:20 (vol%) hexane:chloroform, was eluted using 80:20 (vol%) hexane:chloroform with a 1 mL min^{-1} flow rate, monitored by absorbance at 254 nm. The retention time deviation was 2.9% over 15 injections, over the course of 6 months.

3.3 Results and discussion

3.3.1 Elution of mono-aromatic to tri-aromatic model compounds using a hexane mobile phase: selectivity and stability comparison of a silica-based amino-cyano column and a hypercrosslinked polystyrene column

Currently, silica-based columns using hexane mobile phase are recommended for analyzing middle distillates (boiling point range 150 – 400°C) [1-3]. In this section the objective is to compare selectivity and stability of Partisil 5PAC silica-based column vs. the Chromalite 5HGN hypercrosslinked polystyrene column using the same parameters as in standardized methods for middle distillates with a boiling point range of 150 – 400°C [2,3]. The range of aromatic model compounds, temperature, mobile

phase and silica-based column used in this section are as suggested by the Institute of Petroleum and American Society of Testing and Materials [2,3], and some additional model compounds are included.

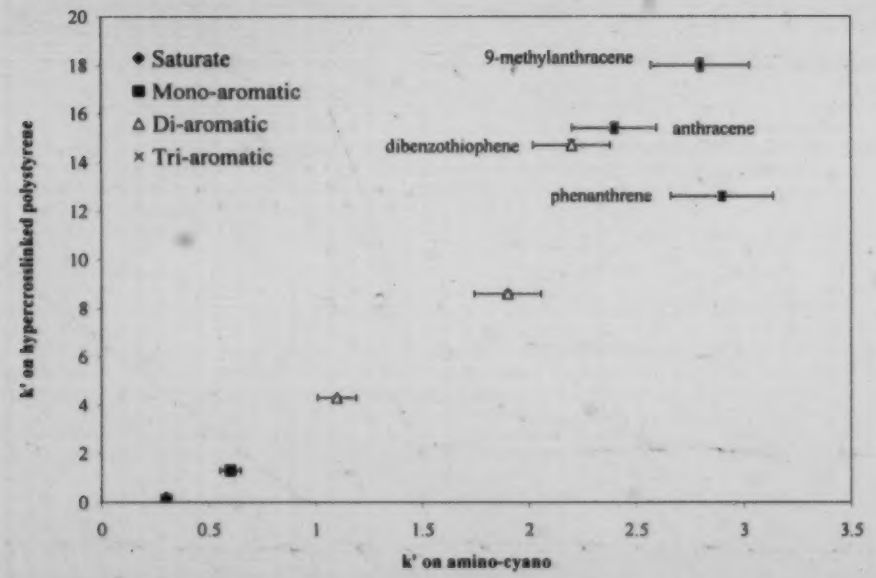

Figure 3.2 Comparison of retention factors (k') of model compounds on silica-based amino-cyano[A] (Partisil 5PAC) and hypercrosslinked polystyrene[B] (Chromalite 5HGN) columns.

A: 4.6 mm ID x 250 mm Partisil 5PAC column, 5 μm particles, hexane mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, refractive index detector

B: 4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, hexane mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 50 μL, refractive index detector

X-error bars are 8.2% and Y-errors bars are 1.4% as per retention time repeatability.

The silica-based amino-cyano and hypercrosslinked polystyrene columns offer different selectivity using a hexane mobile phase, as shown by the plot of $k'_{\text{hypercrosslinked polystyrene}}$ vs. $k'_{\text{amino-cyano}}$ for the same compounds on the two columns (Figure 3.2). The saturates, mono-aromatics and di-aromatics elute in the same order for both columns. With the exception of phenanthrene, the tri-aromatics and dibenzothiophene (a di-aromatic sulphur compound) (Table 3.3 and Figure 3.2) are more strongly retained on the hypercrosslinked polystyrene. Figure 3.2 indicates that hypercrosslinked polystyrene would offer a larger separation between aromatics and

heteroaromatics, which is ideal for the separation of gas oil resins. To test this hypothesis the retention factors (k') and separation efficiencies (N) (Table 3.3) of the model compounds in each column are compared (Section 1.3.1, Equation 1.10 and 1.14).

Table 3.3 Retention factor (k') and separation efficiency (N) of model compounds on silica-based amino-cyano (Partisil 5PAC) and hypercrosslinked polystyrene (Chromalite 5HGN) columns with hexane mobile phase.

Column	Compound	Hydrocarbon group-type	k'	N
amino-cyano[A]	cyclohexane	Saturate	0.3	8170
	o-xylene	Mono-aromatic	0.6	9060
	naphthalene	Di-aromatic	1.1	12570
	9,10-dihydroanthracene	Di-aromatic	1.9	15900
	dibenzothiophene	Di-aromatic sulphur	2.2	15130
	anthracene	Tri-aromatic	2.4	13600
	9-methylanthracene	Tri-aromatic	2.8	13500
	phenanthrene	Tri-aromatic	2.9	11740
hypercrosslinked polystyrene[B]	cyclohexane	Saturate	0.2	770
	o-xylene	Mono-aromatic	1.3	880
	naphthalene	Di-aromatic	4.3	910
	9,10-dihydroanthracene	Di-aromatic	8.6	730
	phenanthrene	Tri-aromatic	12.6	410
	dibenzothiophene	Di-aromatic sulphur	14.7	780
	anthracene	Tri-aromatic	15.4	870
	9-methylanthracene	Tri-aromatic	18.0	680

A: 4.6 mm ID x 250 mm Partisil 5PAC column, 5 μm particles, hexane mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, refractive index detector

B: 4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, hexane mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 50 μL, refractive index detector

From Figure 3.2 and Table 3.3, the hypercrosslinked polystyrene column is better than the amino-cyano column for gas oil resins separation because the

hypercrosslinked polystyrene column retains aromatics (higher k') more than the silica-based amino-cyano column. However, hexane mobile phase resulted in broader peaks with width at half height of up to 5.16 minutes (Appendix A), and therefore an effectively lower separation efficiency in hypercrosslinked polystyrene column (Table 3.3). Despite this, naphthalene and anthracene were baseline resolved with a resolution (R_s, Equation 1.8) value of 7.6.

Hexane was not the best mobile phase for the separation of gas oil resins because of the wider peaks. In addition, the elution time of the largest species must be considered. The largest aromatic compound expected in gas oil resins is pentacene [5]. To predict the elution time of pentacene in a hypercrosslinked polystyrene column using the hexane mobile phase, the retention times of its homologous compounds can be used [23]. For a homologous series of compounds separated in the same column and at the same temperature, the net retention time and volume increases exponentially with the number of carbon atoms in the molecule (Equation 3.1). Based on the retention times for naphthalene and anthracene, pentacene would elute after 491 minutes, which would be a long total analysis time.

It was also desirable to determine which system was the most stable by comparing retention time reproducibility. The method described in Section 3.2.3 yielded a retention time deviation of up to 8.2% for four injections over two days on the amino-cyano column, while only up to 1.4% on the hypercrosslinked polystyrene column (Table 3.4). The larger retention time deviation observed using the silica-based amino-cyano column may be explained by the amount of water in the hexane mobile phase. Some of the silica-based stationary phase may have been deactivated by small amounts of water in the mobile phase [4]. Thus, the use of very dry solvents, such as dehydrated organic solvents kept dry with molecular sieves, is recommended [4,19-21]. It was also possible that the amino-cyano column has not reached equilibrium, since the retention times were observed to steadily increase over time (Appendix B). An improperly equilibrated column could lead to irreproducible retention times [21,22]. The hypercrosslinked polystyrene column, on the other hand, was not as sensitive to water content and is expected to equilibrate faster than the amino-cyano column (Appendix B). Using minimal solvent preparation (Section 3.2.1) and equilibration

time (Section 3.2.3), the hypercrosslinked polystyrene column was more stable than the amino-cyano column.

Table 3.4 Retention time deviation of model compounds on silica-based amino-cyano (Partisil 5PAC) and hypercrosslinked polystyrene (Chromalite 5HGN) columns with hexane mobile phase.

Column	Compound	Average Retention Time (min)	Standard Deviation	% Standard Deviation
amino-cyano[A]	cyclohexane	3.80	0.004	0.1
	o-xylene	4.85	0.02	0.4
	naphthalene	6.19	0.32	5.2
	9,10-dihydroanthracene	8.74	0.67	7.7
	dibenzothiophene	9.72	0.31	3.2
	anthracene	10.16	0.84	8.2
	9-methylanthracene	11.45	0.14	1.2
	phenanthrene	11.64	0.19	1.7
Column	**Compound**	**Average Retention Time (min)**	**Standard Deviation**	**% Standard Deviation**
hypercrosslinked polystyrene[B]	cyclohexane	3.59	0.001	0.04
	o-xylene	6.93	0.005	0.07
	naphthalene	15.76	0.19	1.2
	9,10-dihydroanthracene	28.73	0.09	0.3
	phenanthrene	40.68	0.11	0.3
	dibenzothiophene	47.21	0.68	1.4
	anthracene	49.22	0.22	0.4
	9-methylanthracene	57.00	0.23	0.4

A: 4.6 mm ID x 250 mm Partisil 5PAC column, 5 μm particles, hexane mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, refractive index detector

B: 4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, hexane mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 50 μL, refractive index detector

Compared to an silica-based amino-cyano column, the hypercrosslinked polystyrene column is better for gas oil resins, as it offered higher k' for aromatics. However, hexane was not the optimum mobile phase for the separation of gas oil resins

because of the long analysis time, broad peaks (Appendix B), and smaller separation efficiencies (N) (Table 3.3) observed for the model compounds. It is possible to decrease peak widths and total elution time without sacrificing resolution by adding chloroform to the mobile phase, as shown in Section 3.3.2.

3.3.2 Elution of mono- to tri-aromatic model compounds on a hypercrosslinked polystyrene column using a 80:20 (vol%) hexane:chloroform mobile phase

The separation improved in several ways when the mobile phase used with the hypercrosslinked polystyrene column was changed from hexane (Table 3.3) to 80:20 (vol%) hexane:chloroform (Tables 3.5 and 3.6). The observed peak widths decreased (Appendix C) and separation efficiencies increased (Table 3.3 vs. Table 3.5) using an 80:20 (vol%) hexane:chloroform relative to a hexane mobile phase. The analysis time decreased; the retention time of 9-methylanthracene was reduced from 57.06 minutes to 14.04 minutes (Table 3.6). Based on the retention times of naphthalene and anthracene in 80:20 (vol%) hexane:chloroform, assuming a homologous series elution order [22] (Equation 3.1), pentacene will elute in 55 minutes (compared to 491 minutes in hexane, Section 3.3.1).

Adding chloroform decreased the retention time deviation to 0.5% or less for four injections over two days (Table 3.6), compared to 1.4% for hypercrosslinked polystyrene using hexane and 8.2% for silica-based amino-cyano column in hexane (Table 3.3). This may be explained by the greater solubility of water in chloroform relative to hexane; hexane dissolves 0.011 wt% water at 20°C, compared to chloroform which dissolves 0.0720 wt% water at 23°C [27]. Therefore hexane is more susceptible to water content fluctuation than chloroform, so 80:20 (vol%) hexane:chloroform gives more repeatable retention times. Further, over a period of 6 months (15 injections), the observed retention time deviation for this mobile phase and column was 2.9% (Section 3.2.3). This value was comparable to retention time deviation statistics (2-3%) previously observed for silica and bonded nitrile columns that were tested over a period of 10 months using dehydrated organic solvents kept dry with molecular sieves and with an equilibration of 20 times the column volume [21,28].

Table 3.5 Retention factor (k') and separation efficiency (N) of aromatic model compounds on a hypercrosslinked polystyrene (Chromalite 5HGN) column with an 80:20 (vol%) hexane:chloroform mobile phase.

Compound	Hydrocarbon group-type	k'	N
o-xylene	Mono-aromatic	0.3	1390
naphthalene	Di-aromatic	1.1	2340
9,10-dihydroanthracene	Di-aromatic	1.4	1170
dibenzothiophene	Di-aromatic sulphur	2.9	1240
phenanthrene	Tri-aromatic	3.1	1400
anthracene	Tri-aromatic	3.1	1430
9-methylanthracene	Tri-aromatic	3.3	1200

4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, photodiode array detector monitored at 254 nm

Table 3.6 Retention time deviation of aromatic model compounds on a hypercrosslinked polystyrene (Chromalite 5HGN) column with an 80:20 (vol%) hexane:chloroform mobile phase.

Compound	Average Retention Time (min)	Standard Deviation	% Standard Deviation
o-xylene	4.25	0.010	0.2
naphthalene	6.78	0.036	0.5
9,10-dihydroanthracene	7.85	0.022	0.3
dibenzothiophene	12.99	0.030	0.2
phenanthrene	13.44	0.047	0.4
anthracene	13.49	0.047	0.3
9-methylanthracene	14.04	0.031	0.2

4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, photodiode array detector monitored at 254 nm

3.3.3 Elution order of mono- to penta-aromatic model compounds on a hypercrosslinked polystyrene column using an 80:20 (vol%) hexane:chloroform mobile phase

Gas oil resins can contain up to 5 aromatic rings [5]. Thus, continuing with the same mobile phase (80:20 (vol%) hexane:chloroform), the elution order of mono- to penta-aromatic linear, biphenyl linked-, fused angled- and dihydro-aromatics were determined.

For the linear aromatics, as the number of aromatic rings increases so does the retention factor (k') (Table 3.7). The k' for aromatic compounds increases with the number of rings because π – electrons in larger ring systems are more easily polarized. The retention of aromatic compounds on hypercrosslinked polystyrene column was caused by London dispersion forces (instantaneous induced dipole – induced dipole) [22,24] between the analytes and phenyl groups on the stationary phase. Random disturbances in the distribution of electrons on these neutral molecules create a momentary electrical dipole moment. This dipole polarized the electron cloud in an adjacent molecule, inducing a dipole of opposite polarity in the other molecule and creating an attractive force between the two molecules.

The resolution of linear aromatic compounds under the current conditions was satisfactory, with $R_s > 5$ for all compounds (Table 3.7, Figure 3.3). This was far superior to the gel permeation chromatography method described in Chapter 2 using a Waters HR0.5 styrene-divinylbenzene stationary phase, which was not able to baseline resolve ($R_s \geq 1.5$) all of the linear-aromatics.

For compounds with the same number of rings, elution order was determined by the compound's functional classification, with biphenyl linked- and/or fused angled-aromatics eluting first, followed by linear-aromatics and finally dihydro-aromatics (Table 3.7).

Table 3.7 Elution order of aromatic model compounds on a hypercrosslinked polystyrene (Chromalite 5HGN) column.

Hydrocarbons	Hydrocarbon group-type	k'	R_s[A]
benzene	Linear mono-aromatic	0.3	
tetralin	Dihydro mono-aromatic	0.4	
biphenyl	Biphenyl linked di-aromatic	1.0	
naphthalene	Linear di-aromatic	1.1	5.9
9,10-dihydroanthracene	Dihydro di-aromatic	1.4	
p-terphenyl	Biphenyl linked tri-aromatic	3.0	
phenanthrene	Fused angled tri-aromatic	3.1	
anthracene	Linear tri-aromatic	3.1	6.7
5,12-dihydrotetracene	Dihydro tri-aromatic	4.2	
pyrene	Fused angled tetra-aromatic	5.0	
p-quaterphenyl	Biphenyl linked tetra-aromatic	6.5	
chrysene	Fused angled tetra-aromatic	8.1	
tetracene	Linear tetra-aromatic	9.0	7.3
benzo(e)pyrene	Fused angled penta-aromatic	14.2	
pentacene	Linear penta-aromatic	22.1	6.8

4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, photodiode array detector monitored at 254 nm.

A: The resolution value is calculated based on width at half height (Equation 1.8) and is listed for each compound relative to the next lightest linear compound (for example between benzene and naphthalene).

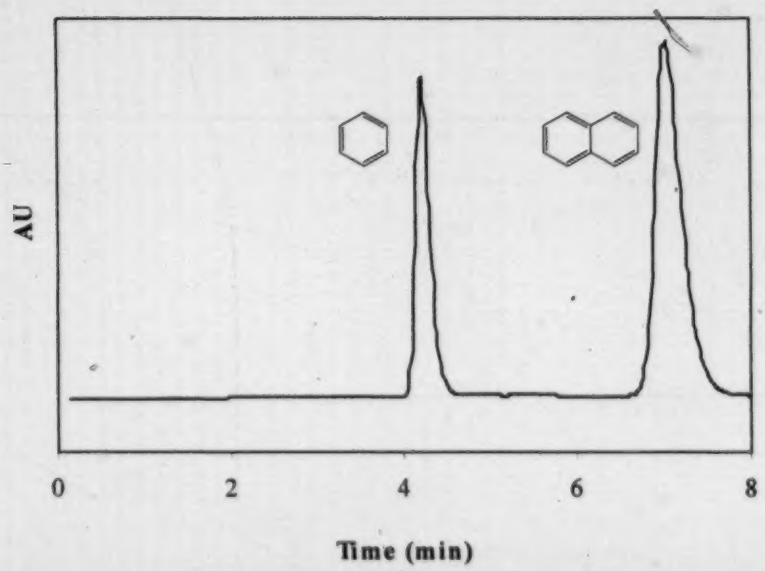

Figure 3.3 Chromatogram of benzene and naphthalene showing a resolution of 5.9.

4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, photodiode array detector monitored at 254 nm.

It is instructive to consider the elution of the aromatic model compounds in terms of homologous series [22]. The homologous series plots of log k' vs. carbon number (n) shown in Figure 3.4 fit to the equation:

$$\log k' = n \times G + F \qquad (3.1)$$

where F and G are constants for a given temperature and stationary phase; G represents the homologous series and F reflects the functional group. For fused angled- and linear-aromatics, the slopes (G = 0.115 ± 0.004) are similar (Figure 3.4), therefore a t-test (Table 3.8) was performed on the individual differences to determine if their slopes were statistically equivalent (Section 1.2, Equations 1.5 and 1.6).

Table 3.8 T-test of linear and fused angled aromatics.

Number of Rings	Number of carbons	Linear log k'	Fused angled log k'	Difference
3	14	0.5	0.5	0
4	18	1.0	0.7	0.3
5	22	1.3	1.2	0.1
			Average Difference	0.13
			Standard Deviation	0.2
			$t_{calculated}$	1.5

4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, photodiode array detector monitored at 254 nm

In this case, d_{ave} was the average difference in log k' between linear and fused angled, and n was the number of pairs of data points (in this case 3). For a 3 sample set with 2 degrees of freedom and 95% confidence interval the t_{table} is 4.303 [25,26]. The $t_{calculated}$ (1.5) was less than the t_{table} (4.303) (Table 3.8) therefore fused angled- or linear- are not statistically different. This indicates that the addition of an aromatic ring in fused angled- or linear- positions are equivalent. According to the t-test, the addition of an aromatic ring, either at fused angled- or linear- position, results in an equivalent increase in log k' [22]. Considering there are only two data points for the dihydro-functionality, the t-test was not performed on it. In comparison, the addition of an aromatic ring via a biphenyl linkage or introducing dihydro-functionality did not give an equivalent slope (G = 0.066 and 0.133 respectively). As for the intercepts (F), all were non-equivalent, since this parameter is unique to each functional group [22].

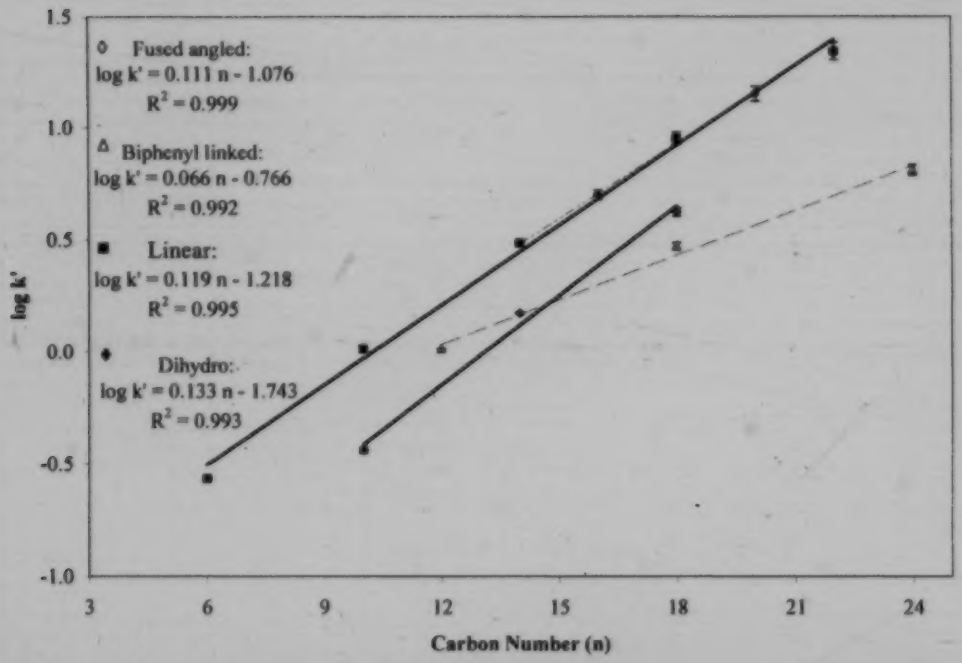

Figure 3.4 Retention statistics for homologous series of aromatic model compounds on a hypercrosslinked polystyrene (Chromalite 5HGN) column using an 80:20 (vol%) hexane:chloroform mobile phase.

4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, photodiode array detector monitored at 254 nm

The error bars of 2.9% are based on the retention time deviation observed for an anthracene quality control sample.

3.3.4 Elution order of di-aromatic sulphur- and nitrogen-containing model compounds on a hypercrosslinked polystyrene column using an 80:20 (vol%) hexane:chloroform mobile phase

In this section it is determined whether di-aromatic compounds containing sulphur and nitrogen [5] (Section 1.1), can be resolved in a hypercrosslinked polystyrene column using an 80:20 (vol%) hexane:chloroform mobile phase.

Among the di-aromatic compounds, naphthalene elutes first, followed by the sulphur- and then the nitrogen-containing model compounds (Table 3.9 and Figures 3.5 and 3.6). This trend maybe explained through intermolecular forces [22,24]. Naphthalene interacts with the stationary phase's phenyl groups through London dispersion forces (induced dipole – induced dipole). The presence of sulphur and nitrogen in the aromatic compounds gives them permanent dipole moments, allowing them to interact with the stationary phase through additional Debye forces (dipole – induced dipole), giving them longer retention times. Nitrogen is more electronegative than sulphur, so the nitrogen-containing aromatic compounds generate stronger Debye forces and are thus retained more strongly than the sulphur compounds.

Table 3.9 Elution order of aromatics, di-aromatic sulphur- and nitrogen-containing model compounds on a hypercrosslinked polystyrene column using an 80:20 (vol%) hexane:chloroform mobile phase.

Hydrocarbons	Retention Time (min)	Sulphur- and nitrogen-containing model compounds	Retention Time (min)[B]	R_s[A]
benzene	4.21			
tetralin	4.51			
biphenyl	6.71			
naphthalene	6.78			
9,10-dihydroanthracene	7.85	phenylsulfide	7.25	
		phenyldisulfide	9.08	1.9
		dibenzothiophene	12.99	3.5
p-terphenyl	13.11			
phenanthrene	13.44			
anthracene	13.49			
5,12-dihydrotetracene	17.21			
pyrene	19.92			
p-quaterphenyl	24.70			
chrysene	30.12	2-naphthalenethiol	29.81	6.0
tetracene	32.93			
		quinoline	39.13	1.3
benzo(e)pyrene	50.21	2-aminonaphthalene	52.42	1.4
pentacene	76.12	carbazole	74.31	1.4

4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, photodiode array detector monitored at 254 nm

A: The resolution value is calculated based on width at half height (Equation 1.8) and is listed for each sulphur- or nitrogen- containing model compound relative to the next lightest sulphur- or nitrogen-containing model compound (for example between phenyl disulfide and dibenzothiophene).

B: Aromatic, di-aromatic sulphur- and nitrogen-containing model compounds listed in the same row are co-eluting.

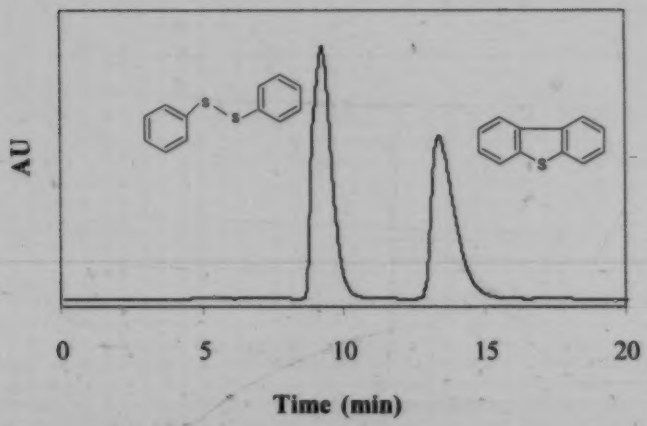

Figure 3.5 Chromatogram of phenyldisulfide and dibenzothiophene showing a resolution of 3.5.

4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, photodiode array detector monitored at 254 nm.

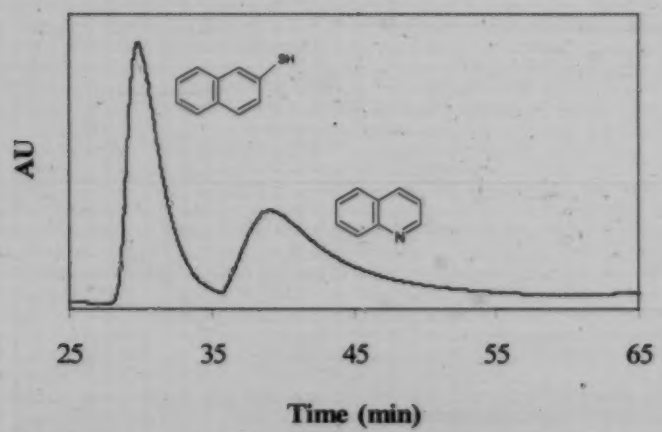

Figure 3.6 Chromatogram of 2-naphthalenethiol and quinoline showing a resolution of 1.3.

4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, photodiode array detector monitored at 254 nm.

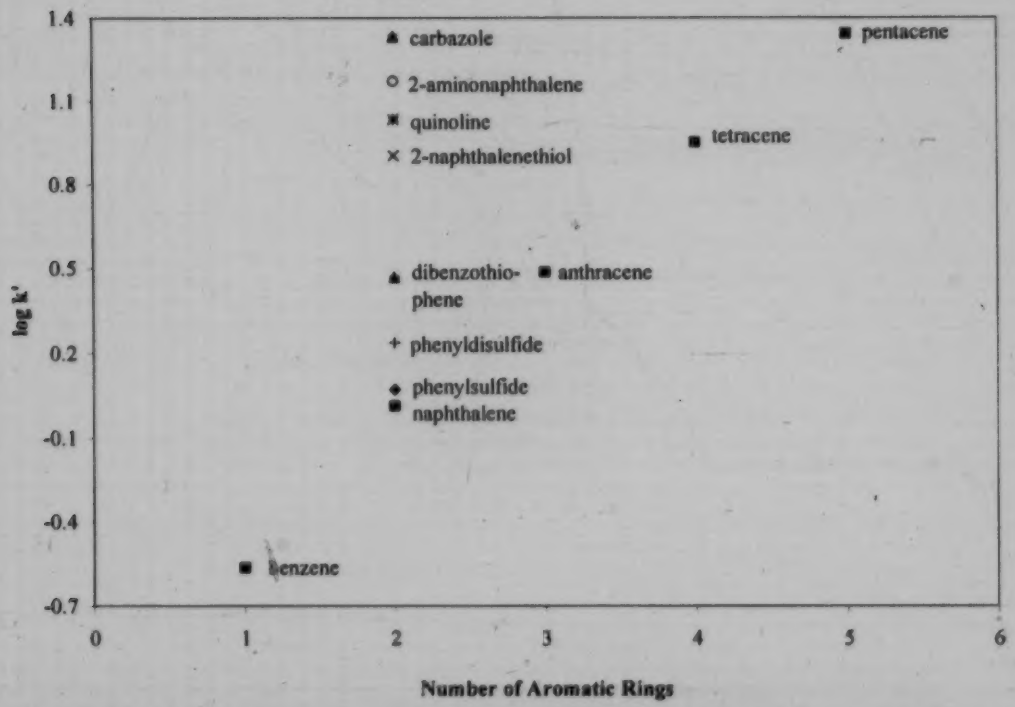

Figure 3.7 Elution order of linear aromatic, di-aromatic sulphur- and nitrogen-containing model compounds on a hypercrosslinked polystyrene column using an 80:20 (vol%) hexane:chloroform mobile phase.

4.6 mm ID x 250 mm hypercrosslinked polystyrene column, 5 μm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, photodiode array detector monitored at 254 nm. The error bars of 2.9% are based on the retention time deviation observed for an anthracene quality control sample.

Several di-aromatic sulphur- and nitrogen-containing model compounds are observed to co-elute with certain linear aromatics (Table 3.9 and Figure 3.7). Phenylsulfide co-elutes with the di-aromatics, dibenzothiophene elutes before the tri-aromatics but is not baseline resolved, and 2-naphthalenethiol co-elutes with the four-ring aromatics. This is consistent with previous observations; for example dibenzothiophene has been observed to elute with tri-aromatics on a silica-based column [5]. For the di-aromatic nitrogen-containing compounds: 2-aminonaphthalene

and carbazole, elute with the five-ring aromatic compounds. Although the sulphur- and nitrogen-containing di-aromatics co-elute with certain linear aromatics it is possible to separate out the different sulphur and nitrogen classes from each other.

Among the di-aromatic sulphur-containing model compounds (Figure 3.7), phenylsulfide elutes first, followed by phenyl disulfide, dibenzothiophene, and then 2-naphthalenethiol. As the number of sulphur compounds increase (from sulfide to disulfide), so does the retention factor (k'). The compounds with a sulphur atom embedded in the ring (i.e. dibenzothiophene) are more retained than the two sulfides. The thiol (-SH) functionality in 2-naphthalenethiol allows it to interact with the stationary phase via hydrogen bonding, giving it the longest retention time. The π - electron system of the stationary phase acts as a weak hydrogen bond acceptor and 2-naphthalenethiol is the proton donor [22,24]. The elution order of the nitrogen-containing di-aromatic compounds is also based on increasing acidity [5], with quinoline (a pyridine, strong base) eluting first, followed by 2-aminonaphthalene (an amine, weak base), and then carbazole (a pyrrole, strong acid). All of the di-aromatics have a resolution range of 1.3 – 6.0 (Table 3.9) between each other. First to elute are the sulfides, followed by thiols and thiophenes. Then the nitrogen class elute in the order of pyridine, amine and finally pyrrole. If the trend continues, this holds promise of separating and determining nitrogen and sulphur distribution in gas oil resins.

3.4 Conclusion

The hypercrosslinked polystyrene column was used with an 80:20 (vol%) hexane:chloroform mobile phase in quasi normal phase chromatography (QNLC) mode to separate a series of aromatic, sulphur- and nitrogen-containing model compounds. The major driving forces for retention in QNLC mode are London dispersion forces, Debye forces, and hydrogen bonding. For linear-aromatic compounds, the retention factor increased with the number of rings contributing to the London dispersion forces. Among compounds of similar molecular weight the general elution order was biphenyl linked- and/or fused angled-aromatic compounds first, followed by linear-aromatic and finally dihydro-aromatic compounds. The sulphur- and nitrogen-containing model

compounds were more strongly retained due to the polarity of the sulphur or nitrogen, which allows these molecules to interact with the stationary phase via Debye interactions, and in some cases hydrogen bonding.

The resolution of linear aromatic compounds (benzene to pentacene) in hypercrosslinked polystyrene using an 80:20 (vol%) hexane:chloroform mobile phase in quasi normal phase chromatography (QNLC) mode resulted in resolutions greater than 5 for all compounds studied. These resolutions were far superior to the gel permeation chromatography method described in Chapter 2 using a Waters HR0.5 styrene-divinylbenzene stationary phase, which was not able to baseline resolve ($R_s \geq 1.5$) all the linear-aromatics. This indicates the great potential of a hypercrosslinked polystyrene column in resolving gas oil resins into its different components.

The hypercrosslinked polystyrene column was able to separate the three classes of sulphur, namely sulfide, thiol and thiophene, as well as separate pyridine, amine and pyrrole. The column has the potential to determine the distribution and relative abundance of sulphur- and nitrogen-containing aromatics in gas oil resins.

In addition, the stability of chromatographic separation results was investigated for two columns: a silica-based amino-cyano column (Partisil 5PAC), and a hypercrosslinked polystyrene column (Chromalite 5HGN). Four injections over two days were made for each set of experimental conditions. The silica amino-cyano based column gave a retention time deviation of 8.2% when a hexane mobile phase was used. For the hypercrosslinked polystyrene column with a hexane mobile phase the retention time deviation was 1.4%, while with the same column using an 80:20 (vol%) hexane:chloroform mobile phase the deviation was only 0.5%. A hypercrosslinked polystyrene column using an 80:20 (vol%) hexane:chloroform mobile phase was therefore the most stable system out of those tested.

A hypercrosslinked polystyrene column was able to resolve all linear aromatics as well as separate the different classes of nitrogen and sulphur aromatics standards. Therefore gas oil resins samples will be run on the hypercrosslinked polystyrene column in Chapter 4.

3.5 References

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Chapter 4: Separation of gas oil resins using high performance liquid chromatography with a hypercrosslinked polystyrene stationary phase

4.1 Introduction

The model compounds chosen to represent components in gas oil resins gave acceptable separation in a hypercrosslinked polystyrene column (Chapter 4). Operating the column in quasi normal phase chromatography (QNLC) mode using an 80:20 (vol%) hexane:chloroform mobile phase resulted in resolutions greater than 5 for linear aromatic compounds (benzene to pentacene). In addition the hypercrosslinked polystyrene column was able to separate the different classes of nitrogen and sulphur aromatics. Therefore gas oil resins samples will be run on the hypercrosslinked polystyrene column.

In this chapter, HPLC using a hypercrosslinked polystyrene stationary phase [1] is applied to the separation of gas oil resins into its different components. The mobile phase and other conditions conducive to the HPLC separation of gas oil resins are determined. The HPLC results are compared to results from aromatic model compounds to determine the possible components present in the gas oil resins. Gas oil resins from the four different sources are compared based on results from HPLC, elemental analysis and nuclear magnetic resonance spectroscopy.

4.2 Experimental

4.2.1 Reagents

Bitumen and heavy oils (Section 1.1) are typically named for their place of origin. The four bitumen samples used in this thesis are from: Athabasca (Athabasca, Alberta, Canada), Wolf Lake (Wolf Lake, Alberta, Canada), Rubiales (Rubiales Field, Colombia) and Kern River (Kern River, California USA).

All solvents were HPLC grade, purchased from Fisher (Ottawa, Ontario, Canada), and were pre-filtered by the manufacturer using 0.5 μm filters. No additional

drying of the solvents was performed. The samples were pre-filtered with a 0.20 μm polytetrafluoroethylene (PTFE)-fluorophore membrane from InnoSep (Samutsakorn, Thailand). Helium (>99.99%) for sparging of the mobile phase (Section 1.2.1) was from BOC Gas (Mississauga, Ontario, Canada).

The aromatic model compounds were chosen based on previous composition studies done on heavy petroleum fractions [2] and the boiling points of each model compound. In addition, all of the model compounds dissolve in hexane (≥ 3 ppm). However they dissolve more readily in the presence of chloroform, therefore the linear and phenyl branched aromatic compounds were dissolved in 80:20 (vol%) hexane:chloroform. The sulphur- and nitrogen-containing model compounds and gas oil resins samples were dissolved in chloroform. Aromatic model compounds were prepared at 0.03 – 20 parts-per-thousand (ppt) concentrations (weight/weight), and the gas oil resins samples were prepared at 0.15 – 0.39 wt%. Tables 4.1 and 4.2 below list the source and purity of each aromatic model compound. The sources were: Sigma-Aldrich (Oakville, Ontario, Canada), Eastman Organic Chemicals (Kingsport, Tennessee, USA), Acros Organics (Morris Plains, New Jersey, USA), ICN Pharmaceuticals (Cleveland, Ohio, USA) and Matheson, Coleman and Bell (Norwood, Ohio, USA).

Gas oil resins for ^{13}C NMR spectroscopy were prepared by mixing 100 mg of material in 600 μL of deuterochloroform ($CDCl_3$ from Fisher Scientific, Ottawa, Ontario, Canada).

Table 4.1 Characteristics of aromatic model compounds.

Compound	Source	Purity	Boiling point (°C) [3]	Absorption maxima (nm) [3-5]
Linear				
benzene	Aldrich	99%	80.1	178
naphthalene	Aldrich	99%	218	268,275,278
anthracene	Aldrich	99%	340	328,345,363
tetracene	Aldrich	98%	U	U
pentacene	Aldrich	U	525	303,351
Phenyl branched				
1-phenyl octane	Aldrich	98%	264	U

U: unspecified

Absorption maxima: Peak absorption wavelengths in each compound's ultraviolet/visible spectrum.

See Figure 1.3 for structures of aromatic model compounds.

Table 4.2 Characteristics of sulphur- and nitrogen-containing model compounds.

Compound	Source	Purity	Boiling point (°C) [3]	Absorption maxima (nm) [3-5]
Sulphur				
Thiol:				
2-naphthalenethiol	Eastman Organic Chemicals	U	286	240,270, 280,290
Sulfide:				
phenyldisulfide	Aldrich	99%	U	U
phenylsulfide	Aldrich	98%	296	U
Thiophene:				
dibenzothiophene	Aldrich	98%	332	237,288
Nitrogen				
Pyrrole:				
carbazole	Acros Organics	96%	355	235,253,296
Pyridine:				
acridine	Aldrich	97%	346	252,354
pyridine	Aldrich	> 99%	115.2	252,352
quinoline	Matheson, Coleman and Bell	U	238	226,230,281
Amine:				
2-aminonaphthalene	Aldrich	U	306	236,269,278, 290,336
Multihetero-aromatic				
acridine orange	ICN	U	U	U
nitrobenzene	Acros Organics	> 95%	210.8	262

U: unspecified

Absorption maxima: Peak absorption wavelengths in each compound's ultraviolet/visible spectrum. See Figure 1.4. for structures of sulphur- and nitrogen-containing model compounds.

4.2.2 Gas oil resins samples

Each bitumen or heavy oil sample was subjected to distillation (one theoretical plate) at reduced pressure between 0.13 and 6.7 kPa (1 and 50 mm Hg) to collect the gas oil fraction (boiling point range 204 – 524°C) (Section 1.1) [6]. The gas oil was then further separated using preparative chromatography [6] (Section 1.1). Each fraction was dried using a rotary evaporator and then weighed to determine the mass composition of the gas oil distillation fraction [6] (Figure 4.1).

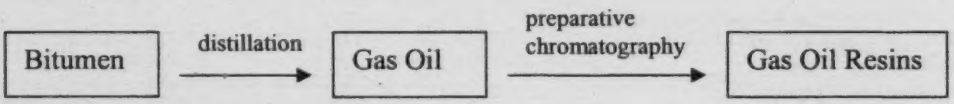

Figure 4.1 Extracting gas oil resins.

High-temperature simulated distillation (HTSD) was performed using an Agilent (Wilmington, Delaware, USA) gas chromatograph and Analytical Control software (Bensalem, Pennsylvania, USA) [7]. A non-polar open tubular (capillary) with 1 μm fused silica film thickness and 0.53 mm ID x 10 m dimensions was used to elute the hydrocarbon components of a sample in order of increasing boiling point. The boiling point range was correlated with the retention times of n-alkanes of known boiling points [7]. The mass of the sample was correlated by calibrating the flame ionization detector's response against a known mass of gas oil sample (BP < 750°C). If an unknown sample contains materials boiling > 750°C, then the flame ionization detector's response is less than the gas oil sample. The difference in response is reported as weight percent material boiling point > 750°C.

The carbon, hydrogen, and nitrogen contents of each gas oil resins sample were determined using a LECO (Mississauga, Ontario, Canada) CHN analyzer [8], and sulphur content was determined using a LECO sulphur analyzer [9]. The CHN analyzer works by introducing the sample into a stream of oxygen which converts carbon to carbon dioxide, hydrogen to water, and nitrogen to nitrogen oxides. Carbon

dioxide and water are then detected in infrared cells, while nitrogen oxide is reduced to nitrogen and detected by a thermal conductivity detector [8]. The sulphur analyzer uses a tube furnace at ≥1350°C with an oxygen-rich atmosphere to convert organic sulphur into sulphur dioxide and sulphur trioxide. These gases (SO_x) are absorbed by dilute hydrogen peroxide to form acidic sulphuric solutions, and the amount of acid is measured to determine the amount of sulphur [9].

The nuclear magnetic resonance (NMR) data for the gas oil resins were analyzed by Dr. Heather Dettman (National Centre for Upgrading Technology in Devon, Alberta, Canada) at room temperature (20 ± 1°C) with a Varian XL-300 NMR spectrometer, operating 75.429 MHz for carbon. The carbon spectra were acquired using an acquisition time of 0.9 seconds, a sweep width of 16,500 Hz, a flip angle of 31.9° and 4 seconds recycle delay [10].

4.2.3 HPLC Apparatus

The HPLC system comprised of a Waters 600 controller multisolvent delivery system (Waters, Milford, Massachusetts, USA and Waters Canada, Mississauga, Ontario, Canada), a Waters 717 autosampler and a Waters 996 multiwavelength photodiode array detector. The data acquisition rate was 1 Hz with a 1 second response time, and data was collected using Empower Pro 2 software from Waters. A column packed with Chromalite 5HGN (Purolite International Limited, Wales, United Kingdom and Bala Cynwyd, Pennsylvania, USA) was used, and the column temperature was controlled using an Eppendorf CH-30 column heater (Mississauga, Ontario, Canada). The Chromalite 5HGN column was packed in-house (Section 3.2.2), in a 4.6mm ID x 250 mm stainless steel column with a 0.5 µm Rheodyne (Rohnert Park, California, USA) frit at each end. Wide bore (0.02 inch or 0.5 mm ID) tubing was used prior to the injector, and narrow bore (0.009 inch or 0.25 mm ID) tubing after the injector.

To test the separation efficiency and peak shape (Section 3.2.2), 5 µL of sample of nitrobenzene (0.5 ppt in 70:20:10 (vol%) hexane:dichloromethane:2-propanol) was injected onto the column (5 µL injection) and eluted at 60°C using a 70:20:10 (vol%)

hexane:dichloromethane:2-propanol mobile phase with a flow rate of 1 mL min^{-1}, and detection at 254 nm [11]. The separation efficiency, N, and USP tailing factor, T_f, were then calculated from the observed retention time and peak width using equations (1.8) and (1.9). Purolite [11] quoted an efficiency of 25,000 plates m^{-1} for nitrobenzene in Chromalite 5HGN column, equivalent to 6,250 plates for the 250 mm column described here. In Chapter 3, the in-house packed Chromalite 5HGN column resulted in an efficiency of 5,000 plates and tailing factor of 1.5 for nitrobenzene. The model compounds were then eluted at 80:20 (vol%) hexane:chloroform (Chapter 3). Then gas oil resins were introduced using the four solvent gradient method. The efficiency of nitrobenzene was checked once more. The efficiency of nitrobenzene decreased to 1800 plates and tailing increased to 2.5. To regenerate the column, tetrahydrofuran was pumped through at 2 mL min^{-1} and 50°C for one hour, then 0.2 mL min^{-1} and 50°C overnight as suggested by Purolite [11]. The nitrobenzene was tested again resulting in an efficiency of 3200 plates and tailing of 1.8. The model compounds were then eluted using the four solvent gradient method, followed by gas oil resins to ensure that the chromatograms were reproducible. The decrease in efficiency may be due to deactivation of the column by the gas oil resins or due to the four solvent gradient (Section 1.3.4.2) [11]. Given that several injections of anthracene (Section 4.3.2.4) did not show a lost of efficiency using the four solvent gradient method, the lost of efficiency is attributed to the gas oil resins.

4.2.4 Method

For HPLC analyses of model compounds and gas oil resins samples, the temperature of the column was maintained at 50 °C with ± 1°C absolute accuracy. The mobile phase flow rate was kept constant at 1.5 mL min^{-1}, and the operating pressure was 500 – 1000 psi depending on the solvent. All solvents were sparged with helium for 15 minutes at 100 mL min^{-1} before use, and then sparged for the remainder of the separation at 50 mL min^{-1}.

In Section 4.3.2.1: Gradient elution of gas oil resins and Section 4.3.2.3: Composition of gas oil resins based on aromatics model compounds, separations were

performed using a four-solvent linear gradient program (see Section 4.3.2.1 for the development of this four-solvent gradient program). The initial mobile phase was 80:20 (vol%) hexane:chloroform, and following sample injection the solvent composition changed linearly over 10 minutes to 100% chloroform. This was followed by a linear gradient over 20 minutes from chloroform to 100% tetrahydrofuran, and then by a linear gradient over 30 minutes from tetrahydrofuran to 100% methanol. After each run, the system was equilibrated by flowing 100% chloroform through the column for 15 minutes, followed by a flow of 80:20 (vol%) hexane:chloroform for 15 minutes.

In Section 4.3.2.2: Testing for irreversible retention of gas oil resins components, chloroform mobile phase was used for the samples eluted without a column. The mobile phase composed of a linear gradient method from chloroform to methanol over 20 minutes for samples eluted using the hypercrosslinked polystyrene column. After each run, the system was equilibrated by flowing 100% chloroform through the column for 15 minutes.

The retention factor (k') defined by equation (1.10) applies only to isocratic separations (i.e. using constant mobile phase composition). In gradient liquid chromatography involving two solvents, a modified retention factor (k*) can be defined which depends not only on the steepness of the gradient, but also on the flow rate and the internal volume of the column [12]. Here, gas oil resins were separated using a four solvent gradient program, making things more complicated, so for simplicity, a retention time window would be quoted for each peak in the chromatogram.

The injection volumes were modified in order to stay within the PDA detector's linear response range of < 2.0 au (Equation 1.20) [13]. In Section 4.3.2.1: Gradient elution of gas oil resins and Section 4.3.2.3: Composition of gas oil resins based on aromatics model compounds, 20 μL volume injections were made. Chromatograms were monitored over a wavelength range of 210 – 400 nm, in which all the aromatic model compounds absorbed. Reference spectra were used to confirm the identities of the model compounds [3-5]. The dwell volume was 12 mL, which corresponds to a dwell time of 8 minutes at 1.5 mL min^{-1} [12]. In Section 4.3.2.2: Testing for

irreversible retention of gas oil resins components, 0.5 μL volumes of gas oil resins solutions were injected.

To determine if all components of the gas oil resins were eluted, the total peak area was compared between chromatograms recorded after gas oil resins injections with and without the column in the system. Using the column, a 20-minute linear gradient elution method from chloroform to methanol was used. When the column was removed from the system, it was replaced by a 25 cm narrow bore (0.009 inch or 0.25 mm ID) stainless steel tube and a chloroform mobile phase was used. Both trials were performed at 50 °C and with a mobile phase flow rate of 1.5 mL min^{-1}.

A quality control sample was analysed periodically to check column stability. Anthracene at 50 parts-per-million (ppm by weight) concentration was eluted with 80:20 (vol%) hexane:chloroform at a 1.5 mL min^{-1} flow rate and monitored by absorbance at 254 nm. The retention time deviation was 0.8% over 10 injections.

4.3 Results and Discussion

4.3.1 Gas oil resins: extraction and boiling point ranges

The products from petroleum refining were characterized based on boiling point range (Section 1.1). The boiling point range for gas oil should be 204 – 524°C. However, the HTSD results in Table 4.3 indicate that the laboratory-scale distillation did not give a sharp boiling point cuts. This was expected for the distillation method used [14].

The saturates, aromatics, and resins contents of the four gas oils are shown in Table 4.4. To determine the boiling point range of gas oil resins an HTSD was performed. The HTSD data for the resins samples in Table 4.5 show that the initial boiling point (IBP) ie. the earliest eluting component of gas oil resins has a boiling point of approximately 300°C. In addition, > 25 wt% of the materials had boiling point > 524°C. With these high boiling points, it becomes even more important to determine if everything was recovered from the HPLC column (Section 4.3.2.2).

Table 4.3 Boiling point range of gas oil.

Name	IBP (°C)	wt% above 524°C	wt% above 750°C
Kern River gas oil	187.6	3	< 1
Rubiales gas oil	205.6	7	< 1
Wolf Lake gas oil	187.6	3	< 1
Athabasca gas oil	200.4	4	< 1

IBP = Initial Boiling Point, ie. the boiling point of the earliest eluting component in gas oil.

As per method ASTM D6352-04e1 [7]

Table 4.4 Weight percent composition of gas oil.

Name	Saturates (wt%)	Aromatics (wt%)	Resins (wt%)
Kern River gas oil	44.7	46.7	8.6
Rubiales gas oil	56.0	40.0	4.0
Wolf Lake gas oil	43.0	53.7	3.3
Athabasca gas oil	41.3	53.4	5.3

As per method ASTM D2007-03 [15].

Table 4.5 Boiling point range of gas oil resins.

Name	IBP (°C)	wt% above 524°C	wt% above 750°C
Kern River gas oil resins	304.2	26	4
Rubiales gas oil resins	304.2	44	8
Wolf Lake gas oil resins	297.6	41	17
Athabasca gas oil resins	293.6	38	14

IBP = Initial Boiling Point, ie. the boiling point of the earliest eluting component in gas oil resins.

As per method ASTM D6352-04e1 [7].

4.3.2 Separation of gas oil resins by HPLC using a hypercrosslinked polystyrene stationary phase

4.3.2.1 Gradient elution of gas oil resins

In literature, group type separations of heavy distillates and coal-derived products are often performed using gradients, back-flushing, valves and multiple columns (Section 1.3.2.3, 1.3.2.4) [16]. For example, Grizzle and Sablotny [17] used amino-bonded silica columns and an n-hexane/dichloromethane gradient mobile phase to separate crude oils and bitumen. Saturated hydrocarbons and aromatic compounds were eluted, and polar (sulfur- and nitrogen-containing) aromatic compounds were back-flushed. Robbins [18] used propylamino-cyano and dinitro-aminopropyl columns and three solvents to analyze a petroleum fraction with boiling points up to 700°C. The mobile phase was composed of n-hexane, dichloromethane, and 2-propanol. Robbins also used valves to direct mobile phase flow between different columns to elute saturates, 1-4 ring aromatics, and polars. In addition, Padlo *et al.* [19] used a gradient mobile phase composed of n-pentane, dichloromethane and 2-propanol to separate coal-derived liquids. By switching the flow through different columns packed with amino-cyano, dinitroaminopropyl-Si and diol-Si stationary phases, saturates, 1-4 ring aromatics and polars were eluted.

The goal is to develop an analytical method that can provide as much chromatographic information from gas oil resins samples as possible, preferably in the shortest amount of time, without needing to back-flush, and using a minimum number of solvents. Gas oil resins were extracted from gas oil using preparative chromatography where 50:50 (vol%) toluene:acetone was used (Section 1.1). Gas oil resins required 100% chloroform to fully dissolve. In Section 3.3.3 an 80:20 (vol%) hexane:chloroform mobile phase was shown to give excellent resolution ($R_s > 5$) for a series of linear aromatic compounds ranging from benzene to pentacene. Therefore a separation using a gradient from 80:20 (vol%) hexane:chloroform to 100% chloroform and finally to 100 vol% methanol was attempted on the Kern River sample. Other experimental conditions included a column temperature at 35°C, a flow rate of

1.0 mL min^{-1} flow rate with 60 minutes gradient time. This three solvent gradient produced a chromatogram containing three peaks (data not shown).

In an attempt to increase the number of peaks, tetrahydrofuran (THF) was introduced. THF is miscible with both hexane and methanol [20], and is one of the most polar normal phase chromatography solvents [12]. When all four solvents were used on Kern River at 35°C, 1.0 mL min^{-1} flow rate with 60 minutes gradient time [12], five peaks appeared in the resulting chromatogram (data not shown). When conditions are further optimized (Section 4.2.4) by increasing the flow rate to 1.5 mL min^{-1}, gradient time of 30 minutes, and increasing the temperature to 50°C (staying below the boiling points of all four solvents), seven chromatographic peaks were obtained in 35 minutes for Kern River gas oil resin (Figure 4.2). The peak assignments will be discussed in detail in Section 4.3.2.3. In comparison to literature [16-19], the method presented in this thesis is much simpler because it requires only one column and no backflushing.

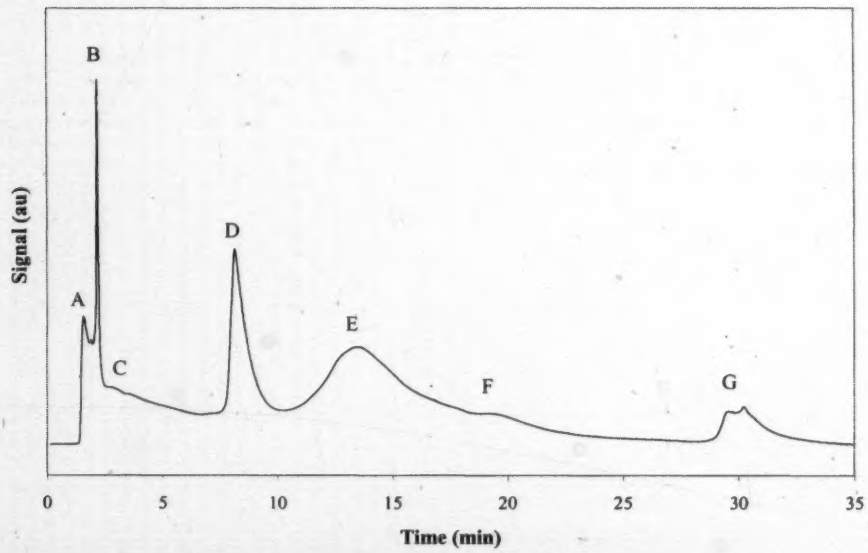

Figure 4.2 Chromatogram of Kern River gas oil resin sample.
4.6 mm ID x 250 mm column, hypercrosslinked polystyrene stationary phase with 5 μm particles, linear gradient method of: 80:20 (vol%) hexane:chloroform to 100% chloroform by 10 minutes, 100% chloroform to 100% tetrahydrofuran by 20 minutes, 100% tetrahydrofuran to 100% methanol by 30 minutes, T = 50°C, F = 1.5 mL min^{-1}, injection volume = 20 μL, absorbance monitored at 300 nm

4.3.2.2 Testing for irreversible retention of gas oil resins components

Seven peaks were observed for Kern River (Section 4.3.2.1). To determine if any components of gas oil resins were irreversibly retained by the hypercrosslinked polystyrene stationary phase, a comparison was made of the total peak area between gas oil resins chromatograms with and without the hypercrosslinked polystyrene column in the system. If everything eluted out of the column, then the area should be constant for each sample because the molar absorptivity (ε) of each analyte and the path length (b) of the detector cell was the same in both trials, while the detector response was linearly related to analyte concentration (c) (Equation 1.20). Table 4.6 compares the peak area for each gas oil resin in the absence (without column area) and presence (with column area) of the hypercrosslinked polystyrene column. There was good agreement between the areas observed with and without the column for each of

the gas oil resins. To determine whether the areas agreed statistically, the observed total peak area was measured for three separations of the Kern River gas oil resin on the hypercrosslinked polystyrene column. A value $t_{calculated}$ was calculated and then compared to a predetermined value appropriate for the specific conditions, t_{table}, to determine whether the change was statistically significant (Section 1.2, Equations 1.1 and 1.2). For a 3-sample set with 2 degrees of freedom and a 95% confidence interval the t_{table} was 4.303 [21,22]. The $t_{calculated}$ (0.24) is less than the t_{table} (4.303) (Table 4.6), indicating that the measured and expected areas agreed within experimental error. Therefore, all components of the Kern River gas oil resin were eluted from the column. Assuming the other samples had similar reproducibility (12%), all components of the gas oil resins eluted out of the column for all four samples.

Table 4.6 Area count of gas oil resins chromatograms.

Gas Oil Resins	Without column area (au)	With column area (au)	Average (au)	Standard deviation (au)
Kern River	3.3×10^6	3.7×10^6	3.2×10^6	0.4×10^6
		2.9×10^6		
		3.1×10^6		
Rubiales	5.4×10^6	5.3×10^6		
Wolf Lake	5.1×10^6	5.0×10^6		
Athabasca	4.7×10^6	4.8×10^6		

4.6 mm ID x 250 mm column, hypercrosslinked polystyrene stationary phase with 5 μm particles, chloroform mobile phase for "without column area", and chloroform to methanol linear gradient method over 20 minutes for "with column area", T = 50°C, F = 1.5 mL min^{-1}, injection volume = 0.5 μL, absorbance monitored at 300 nm

4.3.2.3 Composition of gas oil resins based on aromatic model compounds

Based on Table 4.5, the initial boiling point ie. the boiling point of the earliest eluting component of gas oil resins is 293°C, and the latest eluting component has a

boiling point of >750°C. The peaks in Figure 4.2 were grouped based on the model compounds (Table 4.7). To identify components that may be present in each peak, the aromatic model compounds listed in Tables 4.1 and 4.2 were analyzed using the same chromatographic method. The results are shown in Table 4.7. None of the selected model compounds elute in the vicinity of Peak A. Peak A is essentially the dead time of the column, and so is comprised of unretained components. Peak B eluted at the same time as 1-phenyloctane and benzene. However, gas oil resins could not contain benzene (BP = 80.1°C) or 1-phenyloctane (BP = 264°C) because their boiling points are below the boiling point of the earliest eluting component of gas oil resins (IBP = 293°C, Table 4.5). However, it is likely that Peak B corresponds to alkyl-aromatics as to be in the measured boiling range. Peak C eluted at the same time as naphthalene, phenylsulfide, and phenyldisulfide. Naphthalene (BP = 218°C) has a low boiling point to be in gas oil resins (IBP = 293°C, Table 4.5). But, Peak C may be considered to represent di-aromatic compounds. In addition, as discussed in Section 3.3.4, gas oil resins may contain sulfides. Dibenzothiophene and anthracene have the same retention time as Peak D, suggesting tri-aromatics eluted at this retention time. The model compounds that have equivalent retention times to Peak E include 2-naphthalenethiol, tetracene, quinoline, pyridine, 2-aminonaphthalene, carbazole and pentacene. However, gas oil resins could not contain pyridine (BP = 115°C), unless it had side groups attached. Peak F had the same retention time as acridine and Peak G, the same retention time as acridine orange.

Table 4.7 Aromatic model compounds eluting in each retention time window.

Peak	Retention time window (min)	Model compound	Retention time (min)	Boiling Point (°C) [3]	UV absorptivity (x 10^8 au L $moles^{-1}$)
A	0-2.1	none found			
B	2.1-3.1	(1-phenyloctane)	(2.2)	(264)	
		(benzene)	(3.0)	(80.1)	
C	3.1-7	(naphthalene)	(4.0)	(218)	
		phenylsulfide	4.6	296	8.0
		phenyldisulfide	5.5	U	
D	7-10	dibenzothiophene	7.4	332	7.9
		anthracene	8.0	340	7.6
E	10-18.5	2-naphthalenethiol	11.2	306	6.8
		tetracene	12.0	U	9.8
		quinoline	12.6	238	5.5
		(pyridine)	(13.1)	(115)	
		2-aminonaphthalene	13.8	306	6.1
		carbazole	15.1	355	7.1
		pentacene	16.0	525	
F	18.5-28	acridine	19.5	346	7.7
G	28-40	acridine orange	32.1	U	27

U: unspecified

Brackets indicate model compounds which have the appropriate retention time but are not expected to be in gas oil resins unless they have side-groups attached that increased their boiling points.

4.6 mm ID x 250 mm column, hypercrosslinked polystyrene stationary phase with 5 μm particles, linear gradient method of: 80:20 (vol%) hexane:chloroform to 100% chloroform by 10 minutes, 100% chloroform to 100% tetrahydrofuran by 20 minutes, 100% tetrahydrofuran to 100% methanol by 30 minutes, T = 50°C, F = 1.5 mL min^{-1}, injection volume = 20 μL, absorbance monitored at 300 nm

Besides retention time and boiling point, it was also important to know the UV absorptivity of the aromatic model compound. The UV absorptivity, calculated by dividing the area under the peak in the chromatogram by the concentration of the corresponding compound and measured in units of au L $moles^{-1}$, was equivalent to the product of a compound's molar absorptivity (ε) by the path length (b) of the absorbance detector (Equation 1.20). The observed UV absorptivity for selected aromatic model compounds at a detection wavelength of 300 nm are listed in Table 4.7. The important role the UV absorptivity plays in comparing gas oil resins will be discussed in Section 4.3.3.

4.3.2.4 Sample solvent induced broadening

Both Kern River and model compounds were tested using the same HPLC equipments, column, flow rate, and temperature (Section 1.3.1). However, Kern River peaks (Figure 4.2) were broader than model compound peaks (data not shown). In particular, anthracene in chloroform (t_r = 8.09 min, Table 4.8) and Peak D (t_r = 8.15 min, Table 4.8) of Kern River gas oil resin sample have the same retention time within the allowable 0.8% standard deviation (Section 4.2.4). However, anthracene had an efficiency of 3800, which is six times larger than Peak D efficiency of 580 (Table 4.7). The other sources of peak broadening may be due to either sample solvent induced broadening [23,24] or the presence of more than one component in each peak.

Sample solvent induced broadening occurs when the solvent in which the sample is dissolved is a stronger eluent than the mobile phase [23,24]. In this thesis, the gas oil resins required chloroform to fully dissolve. However, the gradient program started with a less polar mobile phase composed of 80:20 (vol%) hexane:chloroform (Section 4.2.4). Sample solvent induced broadening may present a problem such that at the inlet end of the column the front of the band of sample is in a strong solvent, not highly retained by the column (small k'). After migrating some distance down the column the environment becomes more dilute and the sample's front move faster than the back end of the sample band. This broadens the sample band and is likely to cause peak asymmetry [23,24].

Table 4.8 Kern River vs anthracene retention time and efficiency.

Anthracene		Kern River Peak D	
Retention time (min)	N	Retention time (min)	N
8.09	3800	8.15	580

4.6 mm ID x 250 mm column, hypercrosslinked polystyrene stationary phase with 5 μm particles, linear gradient method of: 80:20 (vol%) hexane:chloroform to 100% chloroform by 10 minutes, 100% chloroform to 100% tetrahydrofuran by 20 minutes, 100% tetrahydrofuran to 100% methanol by 30 minutes, T = 50°C, F = 1.5 mL min^{-1}, injection volume = 20 μL, absorbance monitored at 254 nm for anthracene and 300 nm for Kern River Samples both dissolved in chloroform. One injection for each sample

Table 4.9 Anthracene solvent-induced band broadening.

anthracene in chloroform			anthracene in 80:20 (vol%) hexane:chloroform		
RT (min)	$W_{0.5}$ (min)	N	RT (min)	$W_{0.5}$ (min)	N
8.02	0.27	4900	8.09	0.28	4600
8.03	0.28	4500	8.11	0.33	3350
8.09	0.31	3800	8.18	0.33	3400
8.21	0.31	3900	8.09	0.28	4600
	Average	4280		Average	4000
	Standard Deviation	535		Standard Deviation	720
				S_{pooled}	635
				$t_{calculated}$	0.61

4.6 mm ID x 250 mm column, hypercrosslinked polystyrene stationary phase with 5 μm particles, linear gradient method of: 80:20 (vol%) hexane:chloroform to 100% chloroform by 10 minutes, 100% chloroform to 100% tetrahydrofuran by 20 minutes, 100% tetrahydrofuran to 100% methanol by 30 minutes, T = 50°C, F = 1.5 mL min^{-1}, injection volume = 20 μL, absorbance monitored at 254 nm, sample concentration 50 ppm [weight/weight]

To determine if sample solvent induced band broadening was significant, anthracene in chloroform and anthracene in 80:20 (vol%) hexane:chloroform were run using the four solvent linear gradient method (Section 4.2.4). The efficiency (N) was compared between the two runs because it reflects band broadening (Section 1.3.1, Equation 1.14, and Table 4.9).

To determine whether the results for anthracene in chloroform and anthracene in 80:20 (vol%) hexane:chloroform were statistically different, a t-test [21,22] was performed on the two sets of data (Table 4.9). For comparing replicate measurements from different methods, a value $t_{calculated}$ was computed and then compared to a predetermined value appropriate for the specific conditions, t_{table}, to determine whether the change was statistically significant (Section 1.2, Equations 1.3 and 1.4). For the 4-sample set with 3 degrees of freedom and a 95% confidence interval the t_{table} is 3.182 [21,22]. The $t_{calculated}$ (0.61) is less than the t_{table} (3.182), and therefore the separation efficiencies for anthracene in chloroform and anthracene in 80:20 (vol%) hexane:chloroform were not statistically different. Band broadening due to solvent effects in gas oil resins samples should thus not be an issue.

All the other peaks in the Kern River gas oil resin chromatogram display the same low efficiency (Table 4.10), so it is likely they each correspond to a mixture of components. Given the molecular complexity of petroleum products [2], this was expected.

Table 4.10 Retention time and efficiency for peaks in the chromatogram of the Kern River gas oil resin sample.

Peak	Retention Time (min)	Retention time window (min)	$W_{0.5}$ (min)	Efficiency (N)
A	1.58	0-2.1	U	U
B	2.17	2.1-3.1	0.11	2600
C	3.19	3.1-7	U	U
D	8.15	7-10	0.82	580
E	13.51	10-18.5	4.82	50
F	19.11	18.5-28	U	U
G	30.21	28-40	1.83	1560

U: undetermined due to overlapping peaks

4.6 mm ID x 250 mm column, hypercrosslinked polystyrene stationary phase with 5 μm particles, linear gradient method of: 80:20 (vol%) hexane:chloroform to 100% chloroform by 10 minutes, 100% chloroform to 100% tetrahydrofuran by 20 minutes, 100% tetrahydrofuran to 100% methanol by 30 minutes, T = 50°C, F = 1.5 mL min^{-1}, injection volume = 20 μL, absorbance monitored at 300 nm

4.3.3 Comparison of different gas oil resins

Elemental analysis [8,9], and ^{13}C NMR spectroscopy (Section 4.2.2) data were based on the gas oil resins as a whole. The comparison between the gas oil resins' UV absorptivity (as a whole, Section 4.3.2.2) and their total sulfur, total nitrogen, and NMR spectroscopy aromatic carbon contents are made in Table 4.11. No correlations were found (Figures 4.3 and 4.4).

Table 4.11 HPLC gas oil resins' UV absorptivity and NMR spectroscopy aromatic carbon.

Gas Oil Resins	UV Absorptivity (au ppm^{-1})^{A}	Nitrogen^{B} (wt%)	Sulfur^{B} (wt%)	Aromatic Carbon^{C} (mole %C)
Kern River	2200	2.0	1.0	49.5
Rubiales	1400	1.0	2.0	49.4
Wolf Lake	1550	1.1	3.9	46.9
Athabasca	2140	0.9	4.4	40.8

A: Based on 0.5 µL injections and absorbance at 300 nm.

B: Elemental composition of gas oil resins samples [8,9].

C: NMR spectroscopy [10,25,26].

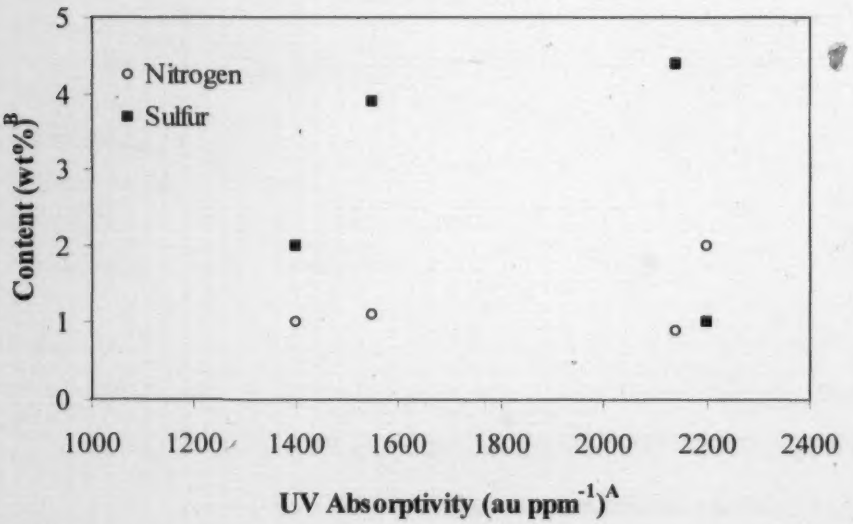

Figure 4.3 Comparison of gas oil resins' UV Absorptivity, elemental sulfur and nitrogen.

A: Based on 0.5 µL injections and absorbance at 300 nm.

B: Elemental composition of gas oil resins samples [8,9].

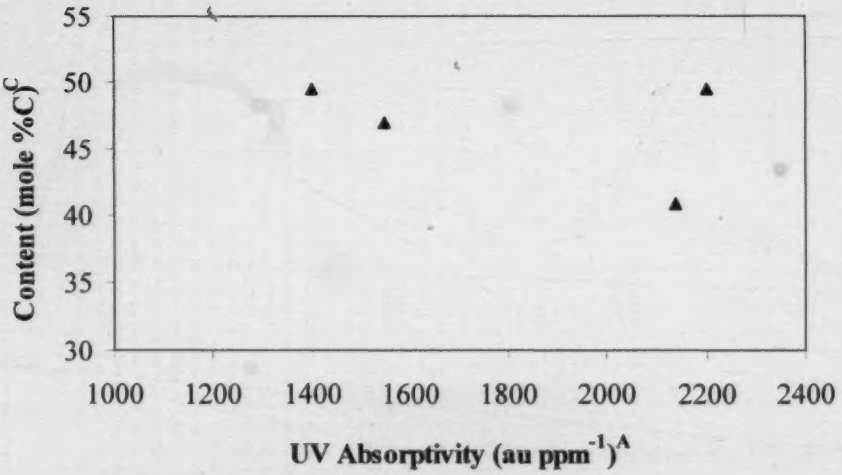

Figure 4.4 Comparison of gas oil resins' UV Absorptivity and aromatic content.

A: Based on 0.5 µL injections and absorbance at 300 nm.

C: NMR spectroscopy [10,25,26].

The UV absorptivity of the gas oil resins were reported as (au ppm^{-1}) because unlike model compounds, the molecular weights of components were unknown. Given that UV absorbance depends upon the molar absorptivities and quantities of molecular species, and molar absorptivity of species varies depending on aromatic carbon, nitrogen, and sulphur type (Table 4.7), it would have been fortuitous if the total contents of aromatic, carbon, sulphur and nitrogen correlated with molar absorptivity of the gas oil resins samples.

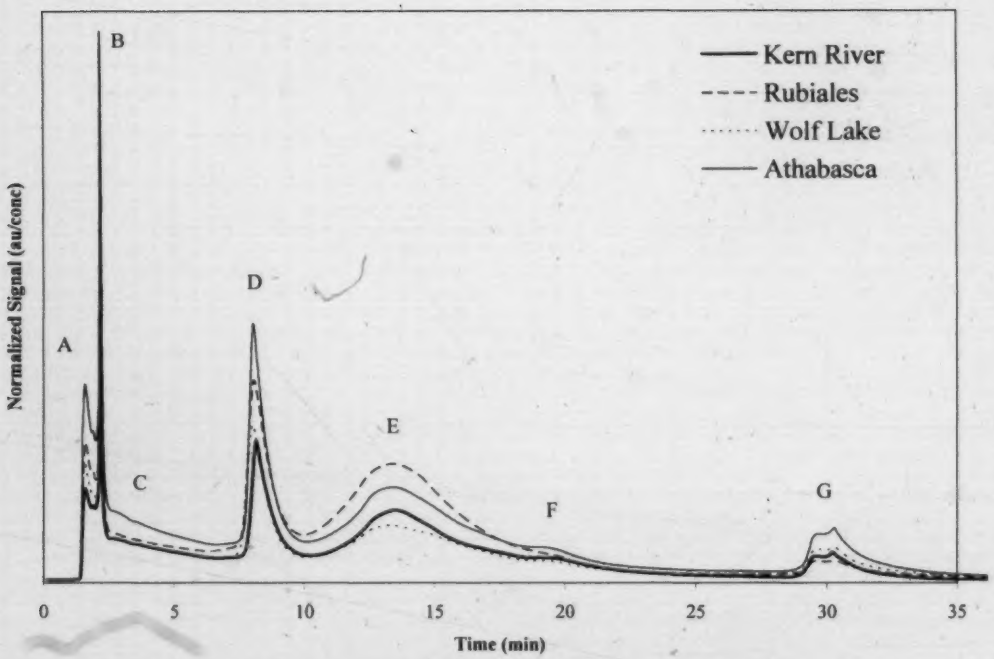

Figure 4.5 Gas oil resins chromatogram, normalized to sample weight.

4.6 mm ID x 250 mm column, hypercrosslinked polystyrene stationary phase with 5 μm particles, linear gradient method of: 80:20 (vol%) hexane:chloroform to 100% chloroform by 10 minutes, 100% chloroform to 100% tetrahydrofuran by 20 minutes, 100% tetrahydrofuran to 100% methanol by 30 minutes, T = 50°C, F = 1.5 mL min^{-1}, injection volume = 20 μL, absorbance monitored at 300 nm

Figure 4.5 shows the chromatograms for all four resins samples normalized to their sample concentration. All four gas oil resins display the same number of peaks (Figure 4.5). However, the order of gas oil resins' intensity varies from peak to peak and differ from the relative absorptivities of the total samples (Table 4.11). For example, for Peak D, Athabasca gives the most intense signal, followed by Rubiales, Wolf Lake and Kern River. However the order in Peak E is Rubiales, Athabasca, Kern River, and Wolf Lake. The HPLC chromatograms indicate differences between the four gas oil resins based on varying peak intensity. For quantification, collections of fractions and characterization is needed.

4.4 Conclusion

Due to limitations of the distillation method, the final boiling points of the gas oil fractions were above 524°C. Consequently, it became even more important to test for irreversible retention of the four gas oil resins; Athabasca, Wolf Lake, Rubiales and Kern River. Results indicated that all components of the gas oil resins completely eluted out of the column for all four samples with 12% reproducibility.

The HPLC method developed in this thesis for the separation gas oil resins was a four-solvent gradient method (hexane, chloroform, tetrahydrofuran and methanol). The flow rate was set to 1.5 mL min^{-1}, the gradient time was 30 minutes, and the column temperature was maintained at 50°C. The samples were separated into seven chromatographic peaks for a total run time of 35 minutes. Based on model compounds, separation was obtained between aromatics, and sulfur- and nitrogen-containing compounds. In addition, the HPLC chromatogram indicated differences between the four gas oil resins based on varying peak intensity. Further quantification of species requires that fractions be collected and further analyzed.

4.5 References

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Chapter 5: Conclusions and Future Directions

5.1 Conclusions

Under conditions tested, the hypercrosslinked polystyrene column (Chapter 3) gave better resolutions than gel permeation chromatography column (Chapter 2) by giving resolutions of greater than five between each linear aromatic model compounds (benzene to pentacene). The hypercrosslinked polystyrene proved to be superior to silica-based columns by offering larger retention factors for the model compounds (Chapter 3). Based on di-aromatic sulfur- and nitrogen-containing model compounds, hypercrosslinked polystyrene was able to separate sulfides, thiols and thiophene; as well as pyridine, amine and pyrrole (Chapter 3). From the model compounds, the hypercrosslinked polystyrene column was deemed able to separate gas oil resins.

Four gas oil resins were analyzed, namely: Athabasca (Athabasca, Alberta, Canada), Wolf Lake (Wolf Lake, Alberta, Canada), Rubiales (Rubiales Field, Colombia), and Kern River (Kern River, California USA), using hypercrosslinked polystyrene column (Chapter 4). All were successfully separated with no irreversibly retained components. The HPLC separation was a four-solvent gradient method (hexane, chloroform, tetrahydrofuran and methanol). The flow rate was set to 1.5 mL min^{-1}, the gradient time was 30 minutes, and the column temperature was maintained at 50°C. The samples were separated into seven chromatographic peaks for a total run time of 35 minutes. In the literature, group type separations of heavy distillates and coal-derived products are often performed using gradients, back-flushing, valves, and multiple columns (Chapter 4). By comparison, the method presented in this thesis is much simpler as it required only one column and no backflushing or valves. Further work will include fraction collection and analysis.

5.2 Future Directions

5.2.1 Gel permeation chromatography

Gel pearmeation chromatography was tested in this thesis. In Chapter 2 GPC using toluene and chloroform as the mobile phases were studied with limited success. Another solvent that could have been tried is tetrahydrofuran. Recently, Sarowha used a tetrahydrofuran mobile phase, two columns with larger pore size, and a higher flow rate (1.5 mL min^{-1}) to separate aromatic compounds at ambient temperature [5]. Styrene-divinylbenzene pore size depends on the solvent and extent of cross-linking for its selectivity of molecular weights [6,7]. Consequently, the pore sizes of styrene-divinylbenzene in tetrahydrofuran would be different than in toluene or chloroform. Sarowha used PL gel 500 Å and PL gel 100 Å pore size (Polymer Laboratories, United Kingdom) columns in series (i.e. using more columns to increase resolution). Aromatic compounds ranging from tri-methyl benzene to coronene (120-276 g mol^{-1} average MW) were separated over a retention volume range of 5.6 mL, and the log [MW] vs. retention volume was linear with a correlation coefficient of $R^2 = 0.995$. The resolution was not stated. It may be worth studying a THF GPC system or even to try the PL gel columns.

5.2.2 Packing hypercrosslinked polystyrene column

Hypercrosslinked polystyrene (Chromalite 5HGN) is not commercially available as a pre-packed column. Therefore, in this thesis (Chapters 3 and 4) hypercrosslinked polystyrene was packed in-house. Purolite [8] quotes 25,000 plates/m efficiency (N). This is equivalent to 6,250 plates (N) for a 250 x 4.6 mm ID hypercrosslinked polystyrene column. Our packing method gave 5,000 plates and the column was not further optimized. In the future, the in house column packing method (Section 3.2.2) could be improved. Kirkland and DeStefano [9] indicated that the smoothness of the stainless steel internal walls was important on column packing.

They stated that the efficiency and stability can be improved by minimizing particles grinding along the column wall. In addition, to ensure column bed stability, it is important to close a freshly packed column quickly [9]. Leaving the column out in the open too long resulted in compressed packed bed springing back to its original non-compressed volume. Then when the column was operated under high pressure or elevated temperatures the packed bed compressed with void or open spaces. Finally, the use of a conical shaped reservoir (40 mL and 27 cm length) [10], would promote a smoother transfer of the slurry to the column, compared to the original reservoir used (170 mL and 8.8 cm length in Section 3.2.2).

5.2.3 Other applications of hypercrosslinked polystyrene

Davankov *et al.* [11] have shown that hypercrosslinked polystyrene can also be used for middle distillates (boiling point range 150 – 400 °C) to separate saturated hydrocarbons, mono-aromatics, di-aromatics and tri-aromatics using 30:20:50 (vol%) hexane-chloroform-2-propanol. Given that hypercrosslinked polystyrene is more stable than a silica-based amino-cyano column (Chapter 3), hypercrosslinked polystyrene may be used to replace a silica-based amino-cyano column for the determination of aromatic hydrocarbon types in middle distillates [12,13]. Extension of their work to include gas oil resins (204 – 524 °C) could be evaluated [14].

Finally, hypercrosslinked polystyrene (Chromalite 5HGN) beads were generously donated by Purolite [15]. Purolite has also donated Purosep-200, which is made up of nitrated hypercrosslinked polystyrene beads. Preliminary studies by Sychov *et al.* [16] indicate that this column may be better than the neutral hypercrosslinked polystyrene at separating polycyclic aromatic hydrocarbons, because nitrogen displays strong pi-electron accepting ability. Consequently, it would be valuable to study Purosep-200.

5.3 References

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Appendix A

As per Table 3.3, average retention time (t_{ave}) and width at half height ($W_{0.5}$) of aromatic standard compounds on silica based amino-cyano (Partisil 5PAC) and hypercrosslinked polystyrene (Chromalite 5HGN) columns with hexane mobile phase.

Column	Compound	Hydrocarbon group-type	t_{ave} (min)	$W_{0.5}$ (min)
amino-cyano[A]	cyclohexane	Saturate	3.80	0.10
	o-xylene	Mono-aromatic	4.85	0.12
	naphthalene	Di-aromatic	6.19	0.13
	9,10-dihydroanthracene	Di-aromatic	8.74	0.16
	dibenzothiophene	Di-aromatic sulfur	9.72	0.19
	anthracene	Tri-aromatic	10.16	0.21
	9-methylanthracene	Tri-aromatic	11.45	0.23
	phenanthrene	Tri-aromatic	11.64	0.25
hypercrosslinked polystyrene[B]	cyclohexane	Saturate	3.59	0.31
	o-xylene	Mono-aromatic	6.93	0.55
	naphthalene	Di-aromatic	15.76	1.23
	9,10-dihydroanthracene	Di-aromatic	28.73	2.51
	phenanthrene	Tri-aromatic	40.68	4.72
	dibenzothiophene	Di-aromatic sulfur	47.21	3.98
	anthracene	Tri-aromatic	49.22	3.93
	9-methylanthracene	Tri-aromatic	57.00	5.16

A: 4.6 mm ID x 250 mm Partisil 5PAC column, 5 μm particles, hexane mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, refractive index detector

B: 4.6 mm ID x 250 mm Hypercrosslinked polystyrene column, 5 μm particles, hexane mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 50 μL, refractive index detector

Appendix B

As per Table 3.4, retention time of aromatic standard componds on silica based amino-cyano (Partisil 5PAC) and hypercrosslinked polystyrene (Chromalite 5HGN) columns with hexane mobile phase.

		Retention Time (min) Day 1		Day 2	
Column	**Compound**	**Inj 1**	**Inj 2**	**Inj 3**	**Inj 4**
amino-cyano[A]	cyclohexane	3.80	3.80	3.80	3.81
	o-xylene	4.83	4.85	4.86	4.87
	naphthalene	6.11	5.95	6.04	6.66
	9,10-dihydroanthracene	8.56	8.23	8.44	9.72
	dibenzothiophene	9.47	9.58	9.66	10.17
	anthracene	9.65	9.75	9.84	11.41
	9-methylanthracene	11.27	11.43	11.54	11.58
	phenanthrene	11.46	11.56	11.65	11.91
Column	**Compound**	**Inj 1**	**Inj 2**	**Inj 3**	**Inj 4**
hypercrosslinked	cyclohexane	3.59	3.59	3.59	3.59
polystyrene[B]	o-xylene	6.93	6.93	6.93	6.94
	naphthalene	15.88	15.48	15.87	15.82
	9,10-dihydroanthracene	28.73	28.63	28.70	28.84
	phenanthrene	40.70	40.78	40.70	40.52
	dibenzothiophene	46.22	47.76	47.53	47.33
	anthracene	49.44	49.28	49.23	48.91
	9-methylanthracene	56.82	57.34	56.91	56.92

A: 4.6 mm ID x 250 mm Partisil 5PAC column, 5 μm particles, hexane mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, refractive index detector

B: 4.6 mm ID x 250 mm Hypercrosslinked polystyrene column, 5 μm particles, hexane mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 50 μL, refractive index detector

Appendix C

As per Table 3.5, average retention time (t_{ave}) and width at half height ($W_{0.5}$) of aromatic standard compounds on a hypercrosslinked polystyrene (Chromalite 5HGN) column with an 80:20 (vol%) hexane:chloroform mobile phase.

Compound	Hydrocarbon group-type	tave (min)	$W_{0.5}$ (min)
o-xylene	Mono-aromatic	4.25	0.27
naphthalene	Di-aromatic	6.78	0.33
9,10-dihydroanthracene	Di-aromatic	7.85	0.54
dibenzothiophene	Di-aromatic sulfur	12.99	0.87
phenanthrene	Tri-aromatic	13.44	0.84
anthracene	Tri-aromatic	13.49	0.84
9-methylanthracene	Tri-aromatic	14.04	0.95

4.6 mm ID x 250 mm Hypercrosslinked polystyrene column, 5 μm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 μL, photodiode array detector monitored at 254 nm.

Appendix D

As per Table 3.6, retention time of aromatic standard compounds on a hypercrosslinked polystyrene (Chromalite 5HGN) column with an 80:20 (vol%) hexane:chloroform mobile phase.

		Retention Time (min) Day 1		Day 2	
Column	**Compound**	**Inj 1**	**Inj 2**	**Inj 3**	**Inj 4**
hypercrosslinked polystyrene	o-xylene	4.24	4.24	4.26	4.26
	naphthalene	6.75	6.75	6.80	6.82
	9,10-dihydroanthracene	7.84	7.84	7.86	7.88
	dibenzothiophene	12.96	12.97	13.02	13.01
	phenanthrene	13.43	13.40	13.43	13.51
	anthracene	13.48	13.43	13.54	13.51
	9-methylanthracene	14.01	14.01	14.07	14.06

4.6 mm ID x 250 mm Hypercrosslinked polystyrene column, 5 µm particles, 80:20 (vol%) hexane:chloroform mobile phase, T = 35°C, F = 1 mL min^{-1}, injection volume = 10 µL, photodiode array detector monitored at 254 nm.